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3D PRINTED TEETH IN ENDODONTICS: WHY, HOW, PROBLEMS AND FUTURE. A NEW PROTOCOL FOR MICRO-CT STUDIES

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3D PRINTED TEETH IN ENDODONTICS: WHY, HOW, PROBLEMS AND FUTURE.

A NEW PROTOCOL FOR MICRO-CT STUDIES

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TIAGO FILIPE DOS SANTOS SOUSA REIS FARIA, DECLARE NO CONFLICTS OF INTEREST RELATED TO THE PRESENT DOCTORAL THESIS

3D PRINTED TEETH IN ENDODONTICS: WHY, HOW, PROBLEMS AND FUTURE. A NEW PROTOCOL FOR MICRO-CT STUDIES

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DEDICATÓRIA

A Cláudia, José y Manuel

¡Son la razón de que esta caminada,
llamada vida, merezca la pena!

A mis Padres

Por el amor incondicional que siempre me han dado

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“ EVERYTHING IS INTERESTING,
IF YOU GO INTO DEEPLY ENOUGH”

RICHARD FEYNMAN

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RESUMO

Tradicionalmente, a norma para estudos ex vivo e cursos preclínicos foi a utilización de dentes humanos extraídos, que durante moito tempo foron considerados como o patrón de ouro (Bitter et al., 2016; De-Deus et al., 2020; Decurcio et al., 2019; Dobros et al., 2022; Hulsmann, 2022; Reymus et al., 2019) e considéranse os mellores para simular o escenario clínico, a pesar da falta de evidencias que apoien esta afirmación (Choi et al., 2021). Non obstante, o emprego de dentes humanos ofrece un medio valioso para reproducir escenarios clínicos e outorga aos estudantes coñecementos sobre a anatomía interna, xunto coa experiencia táctil da manipulación da dentina (Gancedo-Caravia et al., 2020). Non obstante, os dentes humanos extraídos teñen varios inconvenientes, incluíndo a dificultade de recollida, problemas éticos, riscos potenciais de infección cruzada, limitacións de almacenamento e problemas relacionados coa estandarización (Al-Sudani & Basudan, 2017; De-Deus, 2012; Holden & Dracopoulos, 2017; Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020).

Os dentes artificiais que reproducen as características dos dentes naturais poden superar estas limitacións (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Reymus et al., 2021; Reymus et al., 2020). Durante os últimos anos, xurdiron no mercado varios modelos fabricados en fábrica; sendo realistas e estandarizados. Non obstante, os seus custos son elevados, ofrecen un tipo restrinxido de opcións de dentes, hai que ter en conta os prazos de entrega e a dependencia dun fabricante, ademais presentan discrepancias substanciais tanto nos métodos de fabricación como nos materiais entre as marcas comerciais (Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020; Yekta-Michael et al., 2021).

Numerosos estudos empregan dentes impresos en 3D; non obstante, o establecemento de protocolos estandarizados segue sendo fundamental. Entre estes estudos persisten variacións metodolóxicas significativas, o que subliña a necesidade de esforzos de estandarización cohesionados (Decurcio et al., 2019; Hulsmann, 2022). O principal obxectivo detrás do emprego de dentes impresos en 3D é lograr a estandarización. Para os investigadores, isto crea a posibilidade de establecer protocolos iguais e consistentes para estudos ex vivo, permitindo unha comparación sinxela dos resultados. Tendo en conta que a súa anatomía interna debe parecerse moito á dos dentes naturais orixinais, o seu comportamento ante a preparación debe imitar os dentes naturais e deben ser capaces de avaliar e demostrar de forma efectiva as diferenzas entre dous sistemas de preparación, imprimindo tamén estes dentes a gran escala, rápido, e cun custo mínimo debe ser alcanzable.

Polo tanto, para acadar o grao de estandarización necesario e o establecemento de protocolos, é imperativo abordar os problemas asociados aos dentes impresos en 3D.

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Informeuse de dificultades para eliminar o material de soporte de pezas moi detalladas, o que pode provocar un recheo parcial ou completo das canais (Fahad et al., 2013; Hulsmann, 2022; Orel et al., 2021; Reymus et al., 2019). Polo tanto, existe a necesidade de investigar a influencia do material de soporte na actuación do expediente e os seus posibles efectos sobre as variables de interese. O desenvolvemento e validación dun protocolo para a eliminación completa do material de soporte dos dentes impresos en 3D. Posteriormente, é fundamental avaliar a consistencia e precisión da técnica de impresión comparando a anatomía interna dos dentes naturais cos dentes impresos en 3D mediante micro-CT.

Os obxectivos principais da presente tese foron:

1. Avaliar o impacto do material de soporte da réplica TrueTooth® (DELabs), sobre a técnica de preparación e os seus efectos potenciais na avaliación do aumento de volume mediante micro-CT.

2. Desenvolver e validar un protocolo para a eliminación completa do material de soporte dos dentes impresos en 3D e avaliar a súa eficacia comparando a anatomía interna dun dente natural cos dentes impresos en 3D mediante micro-CT.

Ademais, a presente tese tamén ten como obxectivos secundarios:

1. Avaliar o tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular mediante o sistema ProTaper Gold® (Dentsply-Sirona), en TrueTooth® (DELabs) en comparación cos dentes naturais.

2. Avaliar o comportamento do dente natural en comparación cos dentes impresos en 3D, no que se refire á preparación con ProTaper Gold® (Dentsply-Sirona) ata lima F2. Comparando o aumento de volume, o volume de dentina eliminado, os centroides, o transporte, as áreas non preparadas mediante micro-CT.

3. Avaliar o tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular mediante o sistema ProTaper Gold® (Dentsply-Sirona) en dentes naturais en comparación cos dentes impresos en 3D.

4. Avaliar o comportamento dos dentes impresos en 3D á preparación con ProTaper Gold® (Dentsply-Sirona) ata lima F2 e Endogal® (Endogal, Galician Endodontics Company) ata lima D. Comparando o aumento de volume, o volume de dentina eliminado, os centroides, o transporte, as áreas non preparadas mediante micro-CT.

5. Avaliar o tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular nos dentes impresos en 3D mediante o sistema ProTaper Gold® (Dentsply-Sirona) en comparación co sistema Endogal® (Endogal, Galician Endodontics Company).

Para o primeiro obxectivo principal, utilizáronse dentes naturais que cumprisen os criterios de inclusión, dentes con ápices totalmente formados, ausencia de fracturas radiculares, sen signos de reabsorción externa e interna ou tecido cariado na rexión de interese, ausencia de tratamento endodóntico previo, ausencia. de calcificacións, presenza de canais e foramenes apicais separados, e un grao de curvatura que oscila entre 20° e

50°. Avaliouse o grao de curvatura mediante o método de Schneider (Schneider, 1971) e, segundo estes criterios, e para aforrar a asignación de recursos, seleccionáronse as canais bucais de 7 molares maxilares. Ademais, utilizáronse catorce réplicas TrueTooth® (DELabs) (#15-001). TrueTooth® replica #15-001 é un molar maxilar cun canal distobucal cunha curvatura severa e un gran canal mesiovestibular, en forma de cinta, cunha curvatura moderada; estas características son adecuadas para os fins do presente estudo. Os 14 canais mesiovestibulares foron utilizados para avaliar o efecto do material de soporte, mentres que os canais distobucais serviron para a comparación cos 14 canais bucais naturais. Os dous grupos foron escaneados por micro-CT antes da preparación e despois da preparación co sistema ProTaper Gold® (Dentsply-Sirona) ata lima F2. Realizáronse exploracións de micro-CT despois da preparación e avaliouse o aumento de volume, tamén se rexistrou o número de movementos de picoteo necesarios para que cada lima alcance a lonxitude de traballo e a duración da rotación da lima dentro do canal ata alcanzar a lonxitude de traballo.

Para o segundo obxectivo principal, a partir dun conxunto de catorce dentes molares superiores, previamente escaneados por micro-CT, escolleuse un segundo molar maxilar esquerdo que presenta tres raíces totalmente separadas, cada unha presenta un único canal independente sen canais laterales. A raíz mesiovestibular presentaba un canal oval, mentres que a raíz distobucal presentaba un canal redonda de pequeno diámetro e a raíz palatina presentaba un canal redonda de gran diámetro, seleccionouse e xerou un ficheiro STL. O ficheiro STL foi impreso mediante a tecnoloxía PolyJet™ cunha impresora Stratasys Object30 Prime™ (Stratasys Ltd), en modo de alta calidade cun grosor de capa de 16 µm. High Temperature RGD525™ (Stratasys Ltd) foi o material de impresión elixido, xunto co SUP706B™ (Stratasys Ltd) como material de soporte. Despois de realizar o protocolo de eliminación do material de soporte, 20 dentes impresos en 3D foron escaneados por micro-CT antes da preparación e, posteriormente, asignados aleatoriamente en dous grupos (n=10). Un grupo e o dente natural foron preparados con ProTaper Gold® (Dentsply-Sirona), o outro grupo con Endogal® (Endogal, Galician Endodontics Company). Realizáronse exploracións de micro-TC despois da preparación e compáráronse o aumento de volume, o volume de dentina eliminado, os centroides, o transporte e as áreas non preparadas, tamén o número de movementos de picoteo necesarios para que cada lima alcance a lonxitude de traballo e a duración da rotación da lima. rexistráronse a canal ata alcanzar a lonxitude de traballo.

Nos resultados relativos ao primeiro obxectivo primario, os valores medios de aumento de volume foron negativos, respectivamente -1,32 mm^3 e -17,66%.

O tempo necesario para a preparación dos dentes naturais foi lixeiramente maior en comparación cos dentes artificiais, con 64,3 segundos fronte a 63,5 segundos. Non obstante, non se observaron diferenzas estatisticamente significativas entre os dentes naturais e os artificiais en canto ao tempo total de preparación ($p > 0.05$), nin para cada lima individual ($p > 0.05$).

O número de movementos de picoteo necesarios para a preparación dos dentes naturais foi maior en comparación cos dentes artificiais, con 33,5 movementos fronte a 25,86 movementos. Non obstante, non se observaron diferenzas estatisticamente significativas entre os dentes naturais e os artificiais en canto ao número total de movementos de picoteo ($p > 0.05$), nin para cada lima individual ($p > 0.05$).

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Respecto dos resultados asociados ao segundo obxectivo principal, non houbo diferenzas estatisticamente significativas entre o dente natural e os dentes impresos en 3D para todas as variables ($p > 0.05$), sendo estas variables, o volume do canal, o centroide X, Y, Z antes da preparación para tanto os dentes naturais como os dentes impresos en 3D, e a porcentaxe de expansión do volume dos dentes para os dentes impresos en 3D, avaliada mediante exploracións por micro-CT. Estes resultados tiñan como obxectivo avaliar a eficacia do protocolo de retirada do material de soporte.

Os dentes impresos en 3D mostraron un comportamento similar ao dente natural despois da preparación, xa que non houbo diferenzas estatisticamente significativas ($p > 0.05$), relacionadas coas medicións do volume do canal, o centroide X, Y, Z despois da preparación e o volume de dentina. eliminado, porcentaxe de aumento de volume, alteración do centroide X, Y, Z, transporte e porcentaxe de áreas non preparadas.

O tempo necesario para a preparación do dente natural foi lixeiramente maior en comparación cos dentes impresos en 3D, con 179 segundos fronte a 177 segundos. Non obstante, non se observaron diferenzas estatisticamente significativas entre os dentes naturais e os dentes impresos en 3D en canto ao tempo total de preparación ($p > 0.05$) nin para cada lima individual ($p > 0.05$).

O número de movementos de picoteo necesarios para a preparación do dente natural foi maior en comparación cos dentes impresos en 3D, con 102 movementos fronte a 93 movementos. Non obstante, non se observaron diferenzas estatisticamente significativas entre os dentes naturais e os dentes impresos en 3D en canto ao número total de movementos de picoteo ($p > 0.05$) ou para cada lima individual ($p > 0.05$).

Respecto ao aumento de volume, o volume de dentina eliminado, os centroides, o transporte, as áreas non preparadas avaliadas mediante micro-CT e o tempo de preparación e o número de movementos de picoteo necesarios para a preparación do canal radicular, tanto para ProTaper Gold® (Dentsply Sirona) como para Endogal. ® (Endogal, Galician Endodontics Company), xunto co tempo e o número de movementos de picoteo necesarios para cada lima de cada sistema. Non se observaron diferenzas estatisticamente significativas para todas as variables antes da preparación ($p > 0.05$). Ambos sistemas mostraron un comportamento similar, como indica a ausencia de diferenzas estatisticamente significativas ($p > 0.05$) nas medicións do volume do canal, centroide X, Y, Z despois da preparación, volume de dentina eliminado, porcentaxe de aumento de volume, centroide X, Y, Z alteración, transporte e porcentaxe de áreas non preparadas.

Non obstante, o sistema ProTaper Gold® (Dentsply Sirona) demostrou unha maior porcentaxe de aumento de volume e unha menor porcentaxe de áreas non tocadas, nun 47,68% e 54,97%, respectivamente, en comparación co sistema Endogal® (Endogal, Galician Endodontics Company), que mostraron porcentaxes do 45,02% e do 56,41%, respectivamente.

O tempo necesario para a preparación co sistema ProTaper Gold® (Dentsply Sirona) foi maior en comparación co sistema Endogal® (Endogal, Galician Endodontics Company) con 177 segundos fronte a 111,6 segundos. Observáronse diferenzas estatisticamente significativas ($p < 0.05$) entre os dous sistemas en canto ao tempo total de preparación. Ademais, observáronse diferenzas estatisticamente significativas entre a lima

ProGlyder® (Dentsply Sirona) e lima A (Endogal, Galician Endodontics Company) ($p < 0.05$).

O número de movementos de picoteo necesarios para a preparación co sistema ProTaper Gold® (Dentsply Sirona) foi maior en comparación co sistema Endogal® (Endogal, Galician Endodontics Company) con 93 movementos fronte a 47,40 movementos. Observáronse diferenzas estatisticamente significativas entre os dous sistemas en canto ao número de movementos de picoteo ($p < 0.05$). Ademais, observáronse diferenzas estatisticamente significativas entre as limas ProGlyder® (Dentsply Sirona) e A (Endogal, Galician Endodontics Company) ($p < 0.05$).

O primeiro obxectivo principal deste estudo foi avaliar como o material de soporte da réplica TrueTooth® (DELabs) inflúe na técnica de preparación e o seu impacto potencial nos cambios de volume avaliados mediante micro-CT. Os resultados revelaron un valor medio negativo para o aumento do volume do canal radicular despois da preparación no canal mesiovestibular, o que indica unha perda de volume despois da preparación, o que parece absurdo. Tamén se confirmou co canal distobucal que aínda que tiña un valor medio positivo, aínda presentaba valores negativos nalgunhas mostras por aumento de volume.

En consecuencia, é evidente que o material de soporte afecta significativamente á análise 3D. Polo tanto, a hipótese nula é rexeitada. A densidade do material de soporte pospreparación superou a súa densidade previa á preparación e aproximouse á da resina que compoñen a raíz. En consecuencia, despois da preparación do canal, o software de análise 3D xa non distingue estes materiais como entidades separadas, mentres que esa diferenciación era factible antes da preparación.

O obxectivo secundario foi avaliar o tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular mediante o sistema ProTaper Gold® (Dentsply-Sirona), en TrueTooth® (DELabs) en comparación cos dentes naturais. O tempo medio de preparación dos dentes artificiais foi un segundo menos, e foron necesarios menos movementos de picoteo para a preparación do canal en comparación cos dentes naturais. Non obstante, non houbo diferenzas estatisticamente significativas entre eles . Polo tanto, a hipótese nula foi aceptada. Estes achados suxiren que, a pesar de que os dentes artificiais requiren menos tempo e menos movementos de picoteo para a súa preparación, posiblemente debido ás variacións de dureza entre a resina e a dentina humana. (Al-Sudani & Basudan, 2017; Hulsmann, 2022; Reymus et al., 2020).

Os resultados da investigación actual sobre o tempo de preparación e o número de movementos de picoteo necesarios para a preparación do canal radicular suxiren que TrueTooth® (DELabs) é prometedor para o adestramento endodóntico. Non obstante, no futuro son necesarias máis investigacións que empreguen diferentes sistemas de preparación, xeometrías diversas e diferentes movementos de preparación.

O segundo obxectivo principal era describir un protocolo de eliminación de material de soporte e comparar a anatomía do canal radicular entre un dente natural e os dentes impresos en 3D utilizando como referencia as exploracións de Micro-CT do dente natural. A micro-CT, recoñecida pola súa natureza non invasiva e non destrutiva, permite a avaliación de diversos parámetros tanto antes como despois da preparación. É

amplamente considerado como o patrón de ouro na investigación da preparación do canal radicular (Hulsmann, 2022).

Ao comparar o volume e os centroides X, Y, Z do dente natural cos dos dentes impresos en 3D antes da preparación, os nosos resultados non revelaron diferenzas estatisticamente significativas entre eles. Como resultado, aceptouse a hipótese nula. Estes achados indican que o protocolo de eliminación de material de soporte descrito é eficaz para eliminar o material de soporte (SUP706B™) utilizado neste estudo e PolyJet® é axeitado para producir dentes impresos en 3D cunha anatomía interna similar á dos dentes naturais.

Para que os dentes impresos en 3D se utilicen en estudos de preparación ex vivo, o seu comportamento coa preparación debe ser similar ao do dente natural, os resultados actuais sobre o volume de dentina eliminado, a porcentaxe de aumento de volume, o transporte e as áreas non preparadas mostran que non houbo diferenzas estatisticamente significativas entre eles. Polo tanto, a hipótese nula foi aceptada, o que leva á conclusión de que os dentes impresos en 3D se comportan de forma similar aos dentes naturais cando se preparan con ProTaper Gold® (Dentsply-Sirona).

O tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular mediante o sistema ProTaper Gold® (Dentsply-Sirona) en dentes naturais en comparación cos dentes impresos en 3D, mostran que os dentes impresos en 3D requirían menos tempo de preparación e menos movementos de picoteo. Non obstante, non houbo diferenzas estatisticamente significativas entre eles. Polo tanto, aceptouse a hipótese nula, reforzando a conclusión do primeiro obxectivo secundario, de que os dentes impresos en 3D se comportan de forma similar aos dentes naturais cando se preparan con ProTaper Gold® (Dentsply-Sirona).

Avaliar o comportamento dos dentes impresos en 3D á preparación con ProTaper Gold® (Dentsply-Sirona) ata lima F2 e Endogal® (Endogal, Galician Endodontics Company) ata lima D. Isto implicou comparar o aumento de volume, o volume de dentina eliminado, os centroides, o transporte e as áreas non preparadas mediante micro-CT. Os resultados do volume de dentina eliminado, porcentaxe de aumento de volume, transporte e áreas non preparadas mostran que non houbo diferenzas estatisticamente significativas entre os dous sistemas de preparación. Así, aceptouse a hipótese nula.

Non obstante, ao examinar os resultados individualmente, faise evidente que Endogal® (Endogal, Galician Endodontics Company) produciu un menor aumento de volume e eliminación de dentina, xunto cunha maior porcentaxe de áreas non preparadas. A pesar de que ambos os sistemas teñen o mesmo tamaño apical para a preparación, as variacións no deseño de conicidade e sección transversal poderían explicar estas diferenzas. As diferenzas na preparación do conducto radicular tamén están asociadas ás dimensións das limas utilizadas (Chan et al., 2023; Silva et al., 2020).

Para avaliar o tempo e o número de movementos de picoteo necesarios para a preparación do canal radicular nos dentes impresos en 3D mediante o sistema ProTaper Gold® (Dentsply-Sirona) en comparación co sistema Endogal® (Endogal, Galician Endodontics Company), houbo diferenzas estatisticamente significativas entre eles, sendo o Endogal® (Endogal, Galician Endodontics Company) máis rápido e requirindo menos movementos de picoteo. Deste xeito rexeitamos a hipótese nula. Non obstante, estes achados son sinxelos de interpretar e de esperar, xa que o sistema Endogal® (Endogal, Galician

Endodontics Company) utiliza unha lima menos en comparación co sistema ProTaper Gold® (Dentsply-Sirona).

Así mesmo, observáronse diferenzas estatisticamente significativas entre a lima ProGlyder® (Dentsply Sirona) e a lima A (Endogal, Galician Endodontics Company). O tempo de preparación rexistrado foi de 27,30 segundos para a ProGlyder® (Dentsply Sirona) e de 6,90 segundos para a lima A (Endogal, Galician Endodontics Company), mentres que os movementos de picoteo foron de 14,70 e 5,10 respectivamente.

A lima ProGlyder® (Dentsply Sirona) caracterízase por un tamaño de punta de 0,16 mm e unha conicidade variable que oscila entre o 2% e o 8,5% (Alovisi et al., 2017; Gambarini et al., 2021; Keskin et al., 2020; Kirchhoff et al., 2015; Paleker & van der Vyver, 2017). Pola contra, a lima A (Endogal, Galician Endodontics Company) mantén unha conicidade consistente do 3% cun tamaño de punta de 0,15 mm (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021). Aínda que ambas limas comparten un tamaño apical similar, as diferenzas no deseño de sección transversal e conicidade poden explicar estas distincións.

Como se dixo anteriormente, aínda teñen que desenvolverse protocolos para a estandarización dos estudos utilizando dentes impresos en 3D (Hulsmann, 2022), os nosos resultados, dentro das súas limitacións, presentan unha descrición dun protocolo eficaz para a eliminación do material de soporte SUP706B™, demostrando que os dentes impresos en 3D impresos con o material RGD525™ de alta temperatura ten un comportamento similar á preparación de endodoncia que os dentes naturais.
Non obstante, a investigación futura é necesaria para estandarizar os estudos que impliquen dentes impresos en 3D. Isto implica determinar a orientación óptima na cama da impresora para os dentes con varias raíces, decidir o nivel de simplificación para o ficheiro STL sen sacrificar información, avaliar a eficacia do protocolo de eliminación de material de soporte para varias morfoloxías de raíces e comprender como as variacións de rpm, torque, a amplitude dos movementos de picoteo e o deseño da lima poden afectar a preparación dos dentes impresos en 3D.

Na secuencia destes resultados pódese concluír que:

1. Na análise 3D do canal mesiovestibular dos dentes artificiais, o aumento medio do volume do canal radicular resultou negativo, o que indica que o material de soporte influíu na análise 3D Micro-CT da variable en consideración.

2. Non se atoparon diferenzas estatisticamente significativas no tempo e no número de movementos de picoteo entre os dentes naturais e os artificiais. Así, pódese concluír que TrueTooth® serve como unha réplica adecuada con potencial aplicación no adestramento endodóntico.

3. Pódese concluír que o protocolo de eliminación de material de apoio descrito é efectivo para eliminar o material de apoio dos conductos radiculares dos dentes impresos en 3D.

4. A técnica PolyJet™ é adecuada para producir dentes impresos en 3D.

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5. Os dentes impresos en 3D producidos con High Temperature RGD525™ teñen un comportamento similar á preparación endodóntica que o dente natural, e os dentes impresos en 3D pódense usar ao comparar dous sistemas de preparación.

Os dentes impresos en 3D teñen o potencial de converterse no patrón de ouro en estudos ex vivo e adestramento endodóntico, substituíndo aos dentes naturais. Non obstante, para conseguir isto require máis investigación para abordar as limitacións actuais dos dentes impresos en 3D. Ademais, é necesario realizar esforzos concertados para establecer protocolos sólidos, facilitando a estandarización de métodos e permitindo a comparación de resultados entre os estudos.

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RESUMEN

Históricamente, los estudios ex vivo y los cursos preclínicos han favorecido el uso de dientes humanos extraídos, a menudo estimados como el punto de referencia (Bitter et al., 2016; De-Deus et al., 2020; Decurcio et al., 2019; Dobros et al., 2022; Hulsmann, 2022; Reymus et al., 2019) por su capacidad para imitar de cerca las condiciones clínicas, aunque la base empírica de esta afirmación sigue sin demostrarse (Choi et al., 2021). No obstante, el empleo de dientes humanos ofrece un medio valioso para reproducir escenarios clínicos y proporciona a los estudiantes una visión de la anatomía interna, junto con la experiencia táctil de la manipulación de la dentina (Gancedo-Caravia et al., 2020). Sin embargo, la extracción de dientes humanos presenta varios inconvenientes, como la dificultad de la recolección, las preocupaciones éticas, los riesgos potenciales de infección cruzada, las limitaciones de almacenamiento y los problemas relacionados con la estandarización (Al-Sudani & Basudan, 2017; De-Deus, 2012; Holden & Dracopoulos, 2017; Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020).

Los dientes artificiales que reproducen los dientes naturales pueden obviar estos inconvenientes (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Reymus et al., 2021; Reymus et al., 2020). En los últimos años, el mercado ha visto la aparición de varios modelos fabricados en fábrica que presumen de realismo y estandarización. Sin embargo, hay que tener en cuenta su elevado coste, las limitadas opciones de dientes, los largos plazos de entrega y la dependencia de un fabricante concreto, además de que presentan discrepancias sustanciales tanto en los métodos de fabricación como en los materiales de las distintas marcas comerciales (Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020; Yekta-Michael et al., 2021).

Numerosos estudios emplean dientes impresos en 3D; sin embargo, el establecimiento de protocolos estandarizados sigue siendo crucial. Persisten variaciones metodológicas significativas entre estos estudios, lo que subraya la necesidad de esfuerzos de estandarización cohesivos (Decurcio et al., 2019; Hulsmann, 2022). El principal objetivo de emplear dientes impresos en 3D es lograr la estandarización. Para los investigadores, esto crea la posibilidad de establecer protocolos iguales y coherentes para los estudios ex vivo, permitiendo la comparación directa de los resultados. Teniendo en cuenta que su anatomía interna debe parecerse mucho a la de los dientes naturales originales, su comportamiento ante la preparación debe imitar al de los dientes naturales y deben ser capaces de evaluar y demostrar eficazmente las diferencias entre dos sistemas de preparación, también debe ser posible imprimir estos dientes a gran escala, rápidamente y a un coste mínimo.

Por lo tanto, para lograr el grado necesario de normalización y el establecimiento de protocolos, es imprescindible abordar los problemas asociados a los dientes impresos en 3D.

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Se ha informado de la dificultad de retirar el material de soporte de piezas con detalles intrincados, lo que puede dar lugar a un relleno parcial o completo de los canales (Fahad et al., 2013; Hulsmann, 2022; Orel et al., 2021; Reymus et al., 2019). Por lo tanto, existe la necesidad de investigar la influencia del material de soporte en la acción de la lima y sus efectos potenciales sobre las variables de interés. El desarrollo y validación de un protocolo, para la eliminación completa del material de soporte de los dientes impresos en 3D. Posteriormente, es esencial evaluar la consistencia y precisión de la técnica de impresión mediante la comparación de la anatomía interna de los dientes naturales con los dientes impresos en 3D utilizando micro-CT.

Los objetivos principales de la presente tesis fueron:

1. Evaluar el impacto del material de soporte de la réplica TrueTooth® (DELabs), en la técnica de preparación y sus efectos potenciales en la evaluación del aumento de volumen utilizando escáneres micro-CT.

2. Desarrollar y validar un protocolo para la eliminación completa del material de soporte de los dientes impresos en 3D y evaluar su eficacia mediante la comparación de la anatomía interna de un diente natural con dientes impresos en 3D utilizando escáneres micro-CT.

Además, la presente tesis también tiene como objetivos secundarios:

1. Evaluar el tiempo y el número de movimientos de picoteo necesarios para la preparación del conducto radicular utilizando el sistema ProTaper Gold® (Dentsply-Sirona), en TrueTooth® (DELabs) en comparación con los dientes naturales.

2. Evaluar el comportamiento del diente natural en comparación con los dientes impresos en 3D, con respecto a la preparación con ProTaper Gold® (Dentsply-Sirona) hasta la lima F2. Comparando aumento de volumen, volumen de dentina eliminada, centroides, transporte, zonas no preparadas mediante escaneos micro-CT.

3. Evaluar el tiempo y número de movimientos de picoteo necesarios para la preparación del conducto radicular mediante el sistema ProTaper Gold® (Dentsply-Sirona) en diente natural frente a dientes impresos en 3D.

4. Evaluar el comportamiento de dientes impresos en 3D frente a la preparación con ProTaper Gold® (Dentsply-Sirona) hasta la lima F2 y Endogal® (Endogal, Galician Endodontics Company) hasta la lima D. Comparando aumento de volumen, volumen de dentina eliminada, centroides, transporte, zonas no preparadas mediante escaneos micro-CT.

5. Evaluar el tiempo y número de movimientos de picoteo necesarios para la preparación del conducto radicular en dientes impresos en 3D utilizando el sistema ProTaper Gold® (Dentsply-Sirona) comparado con el sistema Endogal® (Endogal, Galician Endodontics Company).

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Para el primer objetivo primario, se utilizaron dientes naturales que cumplieran los criterios de inclusión, dientes con ápices completamente formados, ausencia de fracturas

radiculares, ausencia de signos de reabsorción externa e interna o tejido cariado en la región de interés, ausencia de tratamiento endodóntico previo, ausencia de calcificaciones, presencia de conductos y forámenes apicales separados, y un grado de curvatura que oscilara entre 20° y 50°. El grado de curvatura se evaluó mediante el método de Schneider (Schneider, 1971), y de acuerdo con estos criterios, y para economizar la asignación de recursos, se seleccionaron los conductos bucales de 7 molares maxilares. Asimismo, se utilizaron catorce réplicas TrueTooth® (DELabs) (#15-001). La réplica TrueTooth® #15-001 es un molar maxilar con un conducto distobucal con una curvatura severa y un gran conducto mesiobucal, en forma de cinta, con una curvatura moderada; estas características son adecuadas para los fines del presente estudio. Los 14 conductos mesiobucales se utilizaron para evaluar el efecto del material de soporte, mientras que los conductos distobucales sirvieron para compararlos con los 14 conductos bucales naturales. Los dos grupos fueron escaneados mediante micro-CT antes de la preparación y después de la preparación con el sistema ProTaper Gold® (Dentsply-Sirona) hasta una lima F2. Después de la preparación se realizaron escaneos micro-CT y se evaluó el aumento de volumen, también se registró el número de movimientos de picoteo necesarios para que cada lima alcanzara la longitud de trabajo y la duración de la rotación de la lima dentro del conducto hasta alcanzar la longitud de trabajo.

Para el segundo objetivo primario, de un grupo de catorce molares maxilares, previamente escaneados mediante micro-CT, se eligió un segundo molar maxilar izquierdo con tres raíces completamente separadas, cada una de las cuales presentaba un único conducto independiente sin conductos laterales. La raíz mesiobucal presentaba un conducto ovalado, mientras que la raíz distobucal presentaba un conducto redondo de pequeño diámetro y la raíz palatina presentaba un conducto redondo de gran diámetro. El archivo STL se imprimió utilizando la tecnología PolyJet™ con una impresora Stratasys Object30 Prime™ (Stratasys Ltd), en modo de Alta Calidad que presentaba un grosor de capa de 16 µm. High Temperature RGD525™ (Stratasys Ltd) fue el material de impresión elegido, junto con SUP706B™ (Stratasys Ltd) como material de soporte. Una vez realizado el protocolo de eliminación del material de soporte, se escanearon por micro-CT veinte dientes impresos en 3D antes de la preparación y, a continuación, se asignaron aleatoriamente en dos grupos (n=10). Un grupo y el diente natural se prepararon con ProTaper Gold® (Dentsply-Sirona), el otro grupo con Endogal® (Endogal, Galician Endodontics Company). Después de la preparación se realizaron escaneos micro-CT, y se compararon el aumento de volumen, el volumen de dentina eliminada, los centroides, el transporte y las zonas no preparadas; también se registraron el número de movimientos de picoteo necesarios para que cada lima alcanzara la longitud de trabajo y la duración de la rotación de la lima dentro del conducto hasta alcanzar la longitud de trabajo.

En los resultados relativos al primer objetivo primario, los valores medios de aumento de volumen fueron negativos, respectivamente -1,32 mm^3 y -17,66%.

El tiempo necesario para la preparación de los dientes naturales fue ligeramente superior al de los dientes artificiales, con 64,3 segundos frente a 63,5 segundos. Sin embargo, no se observaron diferencias estadísticamente significativas entre los dientes naturales y los artificiales en cuanto al tiempo total de preparación ($p > 0.05$), o para cada lima individual ($p > 0.05$).

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El número de movimientos de picoteo necesarios para la preparación de los dientes naturales fue superior al de los dientes artificiales, con 33,5 movimientos frente a 25,86 movimientos. Sin embargo, no se observaron diferencias estadísticamente significativas entre los dientes naturales y los artificiales en cuanto al número total de movimientos de picoteo ($p > 0.05$), o para cada lima individual ($p > 0.05$).

En cuanto a los resultados asociados al segundo objetivo primario, no hubo diferencias estadísticamente significativas entre el diente natural y los dientes impresos en 3D para todas las variables ($p > 0.05$), siendo estas variables, el volumen del canal, el centroide X, Y, Z antes de la preparación tanto para el diente natural como para los dientes impresos en 3D, y el porcentaje de expansión del volumen del diente para los dientes impresos en 3D, evaluados mediante escáneres Micro-CT. Estos resultados pretendían evaluar la eficacia del protocolo de eliminación del material de soporte.

Los dientes impresos en 3D mostraron un comportamiento similar al diente natural después de la preparación, ya que no hubo diferencias estadísticamente significativas ($p > 0.05$), relacionadas con las mediciones del volumen del canal, centroide X, Y, Z después de la preparación, y con el volumen de dentina eliminada, porcentaje de aumento de volumen, alteración del centroide X, Y, Z, transporte y porcentaje de áreas no preparadas.

El tiempo necesario para la preparación del diente natural fue ligeramente superior en comparación con los dientes impresos en 3D, con 179 segundos frente a 177 segundos. Sin embargo, no se observaron diferencias estadísticamente significativas entre el diente natural y los dientes impresos en 3D en cuanto al tiempo total de preparación($p > 0.05$), o para cada lima individual($p > 0.05$).

El número de movimientos de picoteo necesarios para la preparación del diente natural fue mayor en comparación con los dientes impresos en 3D, con 102 movimientos frente a 93 movimientos. Sin embargo, no se observaron diferencias estadísticamente significativas entre el diente natural y los dientes impresos en 3D en cuanto al número total de movimientos de picoteo ($p > 0.05$), o para cada lima individual ($p > 0.05$).

En cuanto al aumento de volumen, volumen de dentina removida, centroides, transporte, zonas no preparadas evaluadas mediante micro-CT, y el tiempo de preparación y número de movimientos de picoteo requeridos para la preparación del conducto radicular, tanto para los sistemas ProTaper Gold® (Dentsply Sirona) como Endogal® (Endogal, Galician Endodontics Company), junto con el tiempo y número de movimientos de picoteo requeridos para cada lima de cada sistema. No se observaron diferencias estadísticamente significativas para todas las variables antes de la preparación ($p > 0.05$). Ambos sistemas mostraron un comportamiento similar, como indica la ausencia de diferencias estadísticamente significativas ($p > 0.05$) en las mediciones del volumen del conducto, centroide X, Y, Z tras la preparación, volumen de dentina eliminada, porcentaje de aumento de volumen, alteración del centroide X, Y, Z, transporte y porcentaje de zonas no preparadas.

Sin embargo, el sistema ProTaper Gold® (Dentsply Sirona) demostró un mayor porcentaje de aumento de volumen y un menor porcentaje de zonas no tocadas, con un 47,68% y un 54,97%, respectivamente, en comparación con el sistema Endogal® (Endogal, Galician Endodontics Company), que mostró unos porcentajes del 45,02% y el 56,41%, respectivamente.

El tiempo necesario para la preparación con el sistema ProTaper Gold® (Dentsply Sirona) fue superior en comparación con el sistema Endogal® (Endogal, Galician Endodontics Company) con 177 segundos frente a 111,6 segundos. Se observaron diferencias estadísticamente significativas entre los dos sistemas en cuanto al tiempo total de preparación ($p < 0.05$). Además, se observaron diferencias estadísticamente significativas entre la lima ProGlyder® (Dentsply Sirona) y la lima A (Endogal, Galician Endodontics Company) ($p < 0.05$).

El número de movimientos de picoteo necesarios para la preparación con el sistema ProTaper Gold® (Dentsply Sirona) fue mayor en comparación con el sistema Endogal® (Endogal, Galician Endodontics Company) con 93 movimientos frente a 47,40 movimientos. Se observaron diferencias estadísticamente significativas entre ambos sistemas en cuanto al número de movimientos de picoteo ($p < 0.05$). Además, se observaron diferencias estadísticamente significativas entre la lima ProGlyder® (Dentsply Sirona) y la lima A (Endogal, Galician Endodontics Company) ($p < 0.05$).

El primer objetivo principal de este estudio era evaluar cómo influye el material de soporte de la réplica TrueTooth® (DELabs) en la técnica de preparación y su posible repercusión en los cambios de volumen evaluados mediante escáneres de micro-CT. Los resultados revelaron un valor medio negativo para el aumento de volumen del conducto radicular tras la preparación en el conducto mesiobucal, lo que indica una pérdida de volumen tras la preparación, que parece absurda. También se confirmó con el conducto distobucal que, aunque tenía un valor medio positivo, seguía presentando valores negativos en algunas muestras para el aumento de volumen.

En consecuencia, es evidente que el material de soporte afecta significativamente al análisis 3D. Por lo tanto, se rechaza la hipótesis nula. La densidad del material de soporte tras la preparación superó la densidad previa a la preparación y se aproximó a la de la resina que compone la raíz. Por consiguiente, tras la preparación del conducto, el software de análisis 3D ya no distingue estos materiales como entidades separadas, mientras que dicha diferenciación era factible antes de la preparación.

El objetivo secundario era evaluar el tiempo y el número de movimientos de picoteo necesarios para la preparación del conducto radicular utilizando el sistema ProTaper Gold® (Dentsply-Sirona), sobre TrueTooth® (DELabs) en comparación con los dientes naturales. El tiempo medio de preparación de los dientes artificiales fue un segundo inferior y se necesitaron menos movimientos de picoteo para la preparación del conducto en comparación con los dientes naturales. Sin embargo, no hubo diferencias estadísticamente significativas entre ellos. Por lo tanto, se aceptó la hipótesis nula. Estos resultados sugieren que, a pesar de que los dientes artificiales requieren menos tiempo y menos movimientos de picoteo para la preparación, posiblemente debido a las variaciones de dureza entre la resina y la dentina humana (Al-Sudani & Basudan, 2017; Hulsmann, 2022; Reymus et al., 2020).

Los resultados de la presente investigación en relación con el tiempo de preparación y el número de movimientos de picoteo necesarios para la preparación del conducto radicular sugieren que TrueTooth® (DELabs) es prometedor para la formación en endodoncia. No obstante, en el futuro serán necesarias nuevas investigaciones que empleen distintos

sistemas de preparación, geometrías diversas y movimientos de preparación cinemáticos diferentes.

El segundo objetivo principal era describir un protocolo de eliminación del material de soporte y comparar la anatomía del conducto radicular entre un diente natural y un diente impreso en 3D utilizando escáneres Micro-CT del diente natural como referencia. La Micro-CT, reconocida por su naturaleza no invasiva y no destructiva, permite la evaluación de varios parámetros tanto antes como después de la preparación. Está ampliamente considerado como el patrón oro en la investigación de la preparación del conducto radicular (Hulsmann, 2022).

Al comparar el volumen y los centroides X, Y, Z del diente natural con los de los dientes impresos en 3D antes de la preparación, nuestros resultados no revelaron diferencias estadísticamente significativas entre ellos. En consecuencia, se aceptó la hipótesis nula. Estos resultados indican que el protocolo de eliminación de material de soporte descrito es eficaz para eliminar el material de soporte (SUP706B™) utilizado en este estudio, y PolyJet® es adecuado para producir dientes impresos en 3D con una anatomía interna similar a la de los dientes naturales.

Para que los dientes impresos en 3D puedan ser utilizados en estudios de preparación ex vivo, su comportamiento ante la preparación debe ser similar al diente natural, los presentes resultados en cuanto a volumen de dentina eliminada, porcentaje de aumento de volumen, transporte, y áreas no preparadas, muestran que no hubo diferencias estadísticamente significativas entre ellos. Por lo tanto, se aceptó la hipótesis nula, llegando a la conclusión de que los dientes impresos en 3D se comportan de forma similar al diente natural cuando se preparan con ProTaper Gold® (Dentsply-Sirona).

El tiempo y el número de movimientos de picoteo necesarios para la preparación del conducto radicular mediante el sistema ProTaper Gold® (Dentsply-Sirona) en diente natural en comparación con los dientes impresos en 3D, muestran que los dientes impresos en 3D requirieron menos tiempo de preparación y menos movimientos de picoteo. Sin embargo, no hubo diferencias estadísticamente significativas entre ellos. Por lo tanto, se aceptó la hipótesis nula, reforzando la conclusión del primer objetivo secundario, que los dientes impresos en 3D se comportan de forma similar al diente natural cuando se preparan con ProTaper Gold® (Dentsply-Sirona).

Evaluar el comportamiento de los dientes impresos en 3D a la preparación con ProTaper Gold® (Dentsply-Sirona) hasta la lima F2 y Endogal® (Endogal, Galician Endodontics Company) hasta la lima D. Para ello se comparó el aumento de volumen, el volumen de dentina eliminada, los centroides, el transporte y las zonas no preparadas mediante escáner micro-CT. Los resultados de volumen de dentina removida, porcentaje de aumento de volumen, transporte y áreas no preparadas, muestran que no hubo diferencias estadísticamente significativas entre los dos sistemas de preparación. Así pues, se aceptó la hipótesis nula.

Sin embargo, al examinar los resultados individualmente, se hace evidente que Endogal® (Endogal, Galician Endodontics Company) produjo un menor aumento de volumen y eliminación de dentina, junto con un mayor porcentaje de zonas no preparadas. A pesar de que ambos sistemas tienen el mismo tamaño apical para la preparación, las variaciones en la conicidad y el diseño de la sección transversal podrían explicar estas diferencias.

Las diferencias en la preparación del conducto radicular también están asociadas a las dimensiones de las limas utilizadas (Chan et al., 2023; Silva et al., 2020).

Para evaluar el tiempo y el número de movimientos de picoteo necesarios para la preparación del conducto radicular en dientes impresos en 3D utilizando el sistema ProTaper Gold® (Dentsply-Sirona) en comparación con el sistema Endogal® (Endogal, Galician Endodontics Company), se observaron diferencias estadísticamente significativas entre ambos, siendo el Endogal® (Endogal, Galician Endodontics Company) más rápido y requiriendo menos movimientos de picoteo. De esta forma rechazamos la hipótesis nula. Sin embargo, estos resultados son sencillos de interpretar y esperados, ya que el sistema Endogal® (Endogal, Galician Endodontics Company) utiliza una lima menos en comparación con el sistema ProTaper Gold® (Dentsply-Sirona).

También se observaron diferencias estadísticamente significativas entre la lima ProGlyder® (Dentsply Sirona) y la lima A (Endogal, Galician Endodontics Company). El tiempo de preparación registrado fue de 27,30 segundos para la lima ProGlyder® (Dentsply Sirona) y de 6,90 segundos para la lima A (Endogal, Galician Endodontics Company), mientras que los movimientos de picoteo fueron de 14,70 y 5,10 respectivamente.

La lima ProGlyder® (Dentsply Sirona) se caracteriza por un tamaño de punta de 0,16 mm y una conicidad variable que oscila entre el 2% y el 8,5%(Alovisi et al., 2017; Gambarini et al., 2021; Keskin et al., 2020; Kirchhoff et al., 2015; Paleker & van der Vyver, 2017). Por el contrario, la lima A (Endogal, Galician Endodontics Company) mantiene una conicidad constante del 3% con un tamaño de punta de 0,15 mm (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021). Aunque ambas limas comparten un tamaño apical similar, las diferencias en la conicidad y el diseño de la sección transversal pueden explicar estas distinciones.

Como se dijo anteriormente, los protocolos para la estandarización de los estudios que utilizan dientes impresos en 3D todavía tienen que ser desarrollados (Hulsmann, 2022), nuestros resultados dentro de sus limitaciones presenta una descripción de un protocolo eficaz para la eliminación de material de soporte SUP706B™, demuestran que los dientes impresos en 3D impresos con material de Alta Temperatura RGD525™ tienen un comportamiento similar a la preparación endodóntica que los dientes naturales.

Sin embargo, es necesario investigar en el futuro para estandarizar los estudios con dientes impresos en 3D. Esto implica determinar la orientación óptima en el lecho de la impresora para dientes con múltiples raíces, decidir el nivel de simplificación del archivo STL sin sacrificar información, evaluar la eficacia del protocolo de eliminación del material de soporte para diversas morfologías radiculares y comprender cómo las variaciones en las rpm, el par de torsión, la amplitud de los movimientos de picoteo y el diseño de la lima pueden afectar a la preparación de los dientes impresos en 3D.

En la secuencia de estos resultados, se puede concluir que:

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1. En el análisis 3D del conducto mesiobucal de los dientes artificiales, el aumento medio del volumen del conducto radicular resultó negativo, lo que indica que el material de soporte influyó en el análisis 3D del Micro-CT de la variable considerada.

2. No se encontraron diferencias estadísticamente significativas en el tiempo y el número de movimientos de picoteo entre los dientes naturales y los dientes artificiales. Por lo tanto, se puede concluir que TrueTooth® sirve como una réplica adecuada con potencial aplicación en la formación endodóntica.

3. Se puede concluir que el protocolo de eliminación del material de soporte descrito es eficaz para eliminar el material de soporte de los conductos radiculares de los dientes impresos en 3D.

4. La técnica PolyJet™ es adecuada para producir dientes impresos en 3D.

5. Los dientes impresos en 3D producidos con RGD525™ de alta temperatura tienen un comportamiento similar a la preparación endodóntica que el diente natural, y los dientes impresos en 3D pueden utilizarse cuando se comparan dos sistemas de preparación.

Existe el potencial para que los dientes impresos en 3D establezcan un nuevo estándar en los estudios ex vivo y en la formación endodóntica, suplantando potencialmente a los dientes naturales. Sin embargo, para conseguirlo es necesario seguir investigando para abordar las limitaciones actuales de los dientes impresos en 3D. Además, se necesitan esfuerzos concertados para establecer protocolos robustos, que faciliten la estandarización de métodos y permitan la comparación de resultados entre estudios.

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SUMMARY

Historically, ex vivo studies and pre-clinical courses have favoured the use of extracted human teeth, often esteemed as the benchmark (Bitter et al., 2016; De-Deus et al., 2020; Decurcio et al., 2019; Dobros et al., 2022; Hulsmann, 2022; Reymus et al., 2019) for their ability to closely mimic clinical conditions, although the empirical basis for this assertion remains unsubstantiated (Choi et al., 2021). Nonetheless, employing human teeth offers a valuable means of replicating clinical scenarios and grants students insight into internal anatomy, along with the tactile experience of dentine manipulation (Gancedo-Caravia et al., 2020). However, extracted human teeth has several drawbacks, including the difficulty of collection, ethical concerns, potential risks of cross-infection, storage limitations, and issues regarding standardization (Al-Sudani & Basudan, 2017; De-Deus, 2012; Holden & Dracopoulos, 2017; Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020).

Artificial teeth that reproduce natural teeth may bypass these drawbacks (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Reymus et al., 2021; Reymus et al., 2020). In recent years, the market has seen the emergence of several factory-manufactured models that boast realism and standardization. Nevertheless, their high costs, limited tooth options, extended delivery times, and reliance on a specific manufacturer warrant consideration, also they exhibit substantial discrepancies in both manufacturing methods and materials across commercial brands (Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020; Yekta-Michael et al., 2021).

Numerous studies employ 3D printed teeth; however, the establishment of standardized protocols remains crucial. Significant methodological variations persist among these studies, underscoring the need for cohesive standardization efforts (Decurcio et al., 2019; Hulsmann, 2022). The main objective behind employing 3D printed teeth is achieving standardization. For researchers, this creates the possibility of establishing equal and consistent protocols for ex vivo studies, enabling straightforward comparation of results. Keeping in mind that their internal anatomy should closely resemble that of the original natural teeth, their behaviour to preparation should mimic natural teeth and they should be effectively capable to evaluate and demonstrate differences between two preparation systems, also printing these teeth on a large scale, rapidly, and at minimal cost should be achievable.

Therefore, in order to achieve the requisite degree of standardization and the establishment of protocols, it is imperative to address the problems associated with 3D printed teeth.

Difficulty has been reported in removing support material from intricately detailed parts, potentially resulting in partial or complete filling of canals (Fahad et al., 2013; Hulsmann,

2022; Orel et al., 2021; Reymus et al., 2019). Therefore, there is the need to investigate the influence of support material on the file action and its potential effects on the variables of interest. The development and validation of a protocol, for complete removing support material from 3D printed teeth. Subsequently, assessing the consistency and accuracy of the printing technique by comparing the internal anatomy of natural teeth with 3D printed teeth using micro-CT is essential.

The primary aims of the present thesis were:

1. Evaluate the impact of support material of TrueTooth® (DELabs) replica, on the preparation technique and its potential effects on the volume increase evaluation using micro-CT scans.

2. Develop and validate a protocol for complete removing support material from 3D printed teeth and assessing its efficacy by comparing the internal anatomy of a natural tooth with 3D printed teeth using micro-CT scans.

Additionally, the present thesis also has as secondary aims:

1. Evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, on TrueTooth® (DELabs) compared to natural teeth.

2. Assess the behaviour of natural tooth compared to 3D printed teeth, regarding preparation with ProTaper Gold® (Dentsply-Sirona) up to the F2 file. Comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

3. Evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system on natural tooth compared to 3D printed teeth.

4. Assess the behaviour of 3D printed teeth to preparation with ProTaper Gold® (Dentsply-Sirona) up to F2 file and Endogal® (Endogal, Galician Endodontics Company) up to D file. Comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

5. Evaluate the time and number of pecking movements required for root canal preparation on 3D printed teeth using the ProTaper Gold® (Dentsply-Sirona) system compared to Endogal® (Endogal, Galician Endodontics Company) system.

For the first primary aim, were used natural teeth that met the inclusion criteria, teeth with fully formed apices, absence of root fractures, no signs of external and internal resorption or decayed tissue in the region of interest, absence of previous endodontic treatment, absence of calcifications, presence of separated canals and apical foramens, and a curvature degree ranging between 20° and 50°. The curvature degree was assessed using Schneider's method (Schneider, 1971), and according to these criteria, and to economize resources allocation, the buccal canals of 7 maxillary molars were selected. Also, were used fourteen TrueTooth® (DELabs) replicas (#15-001) were used. TrueTooth® replica #15-001 is a maxillary molar with a distobuccal canal with a severe curvature and a large

mesiobuccal canal, ribbon-shaped, with a moderate curvature; these features are adequate for the purposes of the present study. The 14 mesiobuccal canals were utilized to assess the effect of the support material, while the distobuccal canals served for comparison with the 14 natural buccal canals. The two groups were micro-CT scanned before preparation and after preparation with ProTaper Gold® (Dentsply-Sirona) system until a F2 file. Micro-CT scans were done after preparation, and volume increase was evaluated, also the number of pecking movements required for each file to reach the working length and the duration of file rotation within the canal until reaching the working length were recorded.

For the second primary aim, from a pool of fourteen maxillary molar teeth, previously micro-CT scanned, a second left maxillary molar featuring three fully separated roots, each presenting a single independent canal without any lateral canals was chosen. The mesiobuccal root exhibited an oval canal, while distobuccal root presented a small diameter round canal and palatal root presented a large diameter round canal was selected, and a STL file was generated. The STL file was printed using PolyJet™ technology with a Stratasys Object30 Prime™ printer (Stratasys Ltd), in High-Quality mode featuring a layer thickness of 16 µm. High Temperature RGD525™ (Stratasys Ltd) was the printing material chosen, along with SUP706B™ (Stratasys Ltd) as the support material. After the support material removal protocol were done, twenty 3D printed teeth were micro-CT scanned before preparation, and thereafter randomly assigned into two groups (n=10). One group and the natural tooth were prepared using ProTaper Gold® (Dentsply-Sirona), the other group with Endogal® (Endogal, Galician Endodontics Company). Micro-CT scans were done after preparation, and volume increase, volume of dentin removed, centroids, transportation, and unprepared areas were compared, also the number of pecking movements required for each file to reach the working length and the duration of file rotation within the canal until reaching the working length were recorded.

In the results regarding to the first primary aim, the mean values for volume increase were negative, respectively -1.32 mm^3 and -17.66%.

The time required for preparation of natural teeth was slightly higher compared to artificial teeth, with 64.3 seconds versus 63.5 seconds. However, no statistically significant differences were observed between natural teeth and artificial teeth in terms of the total time of preparation ($p > 0.05$), or for each individual file ($p > 0.05$).

The number of pecking movements required for preparation of natural teeth was higher compared to artificial teeth, with 33.5 movements versus 25.86 movements. However, no statistically significant differences were observed between natural teeth and artificial teeth in terms of the total number of pecking movements ($p > 0.05$), or for each individual file ($p > 0.05$).

Regarding the results associated to the second primary aim, there were no statistically significant differences between natural tooth and 3D printed teeth for all variables ($p > 0.05$), being these variables, the canal volume, centroid X, Y, Z before preparation for both natural tooth and 3D printed teeth, and percentage of tooth volume expansion for the 3D printed teeth, assessed by Micro-CT scans. These results aimed to evaluate the effectiveness of the support material removal protocol.

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The 3D printed teeth showed a similar behaviour to natural tooth after preparation, since there were no statistically significant differences ($p > 0.05$), related to the measurements

of canal volume, centroid X, Y, Z after preparation, and to volume of dentin removed, percentage of volume increase, centroid X, Y, Z alteration, transportation, and percentage of unprepared areas.

The time required for preparation of natural tooth was slightly higher compared to 3D printed teeth, with 179 seconds versus 177 seconds. However, no statistically significant differences were observed between natural tooth and 3D printed teeth in terms of the total time of preparation ($p > 0.05$), or for each individual file ($p > 0.05$).

The number of pecking movements required for preparation of natural tooth was higher compared to 3D printed teeth, with 102 movements versus 93 movements. However, no statistically significant differences were observed between natural tooth and 3D printed teeth in terms of the total number of pecking movements ($p > 0.05$), or for each individual file ($p > 0.05$).

Regarding the volume increase, volume of dentin removed, centroids, transportation, unprepared areas assessed by micro-CT scans, and the time of preparation and number of pecking movements required for root canal preparation, for both ProTaper Gold® (Dentsply Sirona) and Endogal® (Endogal, Galician Endodontics Company) systems, along with the time and number of pecking movements required for each file of each system. There were no statistically significant differences observed for all variables before preparation ($p > 0.05$). Both systems exhibited similar behaviour, as indicated by the absence of statistically significant differences ($p > 0.05$) in measurements of canal volume, centroid X, Y, Z after preparation, volume of dentin removed, percentage of volume increase, centroid X, Y, Z alteration, transportation, and percentage of unprepared areas.

However, the ProTaper Gold® (Dentsply Sirona) system demonstrated a higher percentage of volume increase and a lower percentage of untouched areas, at 47.68% and 54.97%, respectively, compared to the Endogal® (Endogal, Galician Endodontics Company) system, which showed percentages of 45.02% and 56.41%, respectively.

The time required for preparation with ProTaper Gold® (Dentsply Sirona) system was higher compared to Endogal® (Endogal, Galician Endodontics Company) system with 177 seconds versus 111.6 seconds. Statistically significant differences were observed between the two systems regarding the total time of preparation ($p < 0.05$). Additionally, statistically significant differences were observed between the ProGlyder® (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company) ($p < 0.05$).

The number of pecking movements required for preparation with ProTaper Gold® (Dentsply Sirona) system was higher compared to Endogal® (Endogal, Galician Endodontics Company) system with 93 movements versus 47.40 movements. Statistically significant differences were observed between the two systems regarding the number of pecking movements ($p < 0.05$). Additionally, statistically significant differences were observed between the ProGlyder® (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company) ($p < 0.05$).

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The first primary aim of this study was to assess how the support material of the TrueTooth® (DELabs) replica influences the preparation technique and its potential impact on volume changes evaluated through micro-CT scans. The findings revealed a

negative mean value for root canal volume increase post-preparation in mesiobuccal canal, indicating a loss of volume after preparation, which appears absurd. It was also confirmed with the distobuccal canal that although it had a positive mean value, it still exhibited negative values in some samples for volume increase.

Consequently, it is evident that the support material significantly affects the 3D analysis. Hence, the null hypothesis was rejected. The density of the support material post-preparation exceeded its pre-preparation density and approached that of the resin composing the root. Consequently, following canal preparation, the 3D analysis software no longer distinguishes these materials as separate entities, whereas such differentiation was feasible prior to preparation.

The secondary aim was to evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, on TrueTooth® (DELabs) compared to natural teeth. The mean time of preparation for artificial teeth was one second less, and fewer pecking movements were required for canal preparation compared to natural teeth. However, there were no statistically significant differences between them. Hence, the null hypothesis was accepted. These findings suggest that despite artificial teeth requiring less time and fewer pecking movements for preparation, possibly due to variations in hardness between resin and human dentin (Al-Sudani & Basudan, 2017; Hulsmann, 2022; Reymus et al., 2020).

The findings from the current research concerning preparation time and the number of pecking movements required for root canal preparation, suggest that TrueTooth® (DELabs) holds promise for endodontic training. Nonetheless, further investigations employing different preparation systems, diverse geometries, and different kinematics preparation movements are needed in the future.

The sencond primary aim was to describe a support material removal protocoland and to compare root canal anatomy between a natural tooth and 3D-printed teeth using Micro-CT scans of the natural tooth as a reference. Micro-CT, recognized for its non-invasive and non-destructive nature, enables the assessment of various parameters both pre- and post-preparation. It is widely regarded as the gold standard in root canal preparation research (Hulsmann, 2022).

Upon comparing the volume and centroids X, Y, Z of the natural tooth with those of the 3D printed teeth prior to preparation, our results revealed no statistically significant differences between them. As a result, the null hypothesis was accepted. These findings indicate that the described support material removal protocol is effective in eliminating the support material (SUP706B™) utilized in this study, and PolyJet® is suitable for producing 3D printed teeth with internal anatomy similar to that of natural teeth.

In order 3D printed teeth to be used in ex vivo preparation studies, its behaviour to preparation should be similar to natural tooth, the present results regarding volume of dentin removed, percentage of volume increase, transportation, and unprepared areas, show that there were no statistically significant differences between them. Hence, the null hypothesis was accepted, leading to the conclusion that 3D printed teeth behave similarly to natural tooth when prepared with ProTaper Gold® (Dentsply-Sirona).

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The time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system on natural tooth compared to 3D printed teeth, show that 3D printed teeth required less preparation time and less pecking movements. However, there were no statistically significant differences between them. Hence, the null hypothesis was accepted, reinforcing the conclusion of the first secondary aim, that 3D printed teeth behave similarly to natural tooth when prepared with ProTaper Gold® (Dentsply-Sirona).

To assess the behaviour of 3D printed teeth to preparation with ProTaper Gold® (Dentsply-Sirona) up to F2 file and Endogal® (Endogal, Galician Endodontics Company) up to D file. This involved comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans. Results of volume of dentin removed, percentage of volume increase, transportation, and unprepared areas, show that there were no statistically significant differences between the two preparation systems. Thus, the null hypothesis was accepted.

However, upon examining the results individually, it becomes evident that Endogal® (Endogal, Galician Endodontics Company) resulted in less volume increase and dentin removal, along with a higher percentage of unprepared areas. Despite both systems having the same apical size for preparation, variances in taper and cross-section design could account for these differences. Differences in root canal preparation are also associated with the dimensions of the files utilized (Chan et al., 2023; Silva et al., 2020).

To assess the time and number of pecking movements necessary for root canal preparation on 3D printed teeth using the ProTaper Gold® (Dentsply-Sirona) system compared to Endogal® (Endogal, Galician Endodontics Company) system, there was statistically significant differences between them, being the Endogal® (Endogal, Galician Endodontics Company) faster and requiring fewer pecking movements. In this way the null hypothesis was rejected. However, these findings are straightforward to interpret and expected, as the Endogal® (Endogal, Galician Endodontics Company) system utilizes one less file compared to the ProTaper Gold® (Dentsply-Sirona) system.

Also, statistically significant differences were observed between the ProGlyder® file (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company). The preparation time recorded was 27.30 seconds for the ProGlyder® (Dentsply Sirona) and 6.90 seconds for the A file (Endogal, Galician Endodontics Company), while the pecking movements were 14.70 and 5.10 respectively.

The ProGlyder® file (Dentsply Sirona) is characterized by a tip size of 0.16 mm and a variable taper ranging between 2% and 8.5% (Alovisi et al., 2017; Gambarini et al., 2021; Keskin et al., 2020; Kirchhoff et al., 2015; Paleker & van der Vyver, 2017). In contrast, the A file (Endogal, Galician Endodontics Company) maintains a consistent 3% taper with a tip size of 0.15 mm (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021). Although both files share a similar apical size, differences in taper and cross-section design may explain these distinctions.

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As said before, protocols for standardization of studies using 3D printed teeth still have to be developed (Hulsmann, 2022), our results within its limitations presents a description of an effective protocol for support material SUP706B™ removal, demonstrate that 3D

printed teeth printed with High Temperature RGD525™ material have similar behaviour to endodontic preparation that natural teeth.

However, future research is necessary to standardize studies involving 3D printed teeth. This entails determining the optimal orientation on the printer bed for multi-rooted teeth, deciding the level of simplification for the STL file without sacrificing information, assessing the effectiveness of support material removal protocol for various root morphologies, and understanding how variations in rpm, torque, amplitude of pecking movements, and file design can impact the preparation of 3D printed teeth.

In the sequence of these results, it can be concluded that:

1. In the 3D analysis of the artificial teeth's mesiobuccal canal, the average increase in root canal volume was found to be negative, indicating that the support material influenced the Micro-CT 3D analysis of the variable under consideration.

2. No statistically significant differences were found in the time and number of pecking movements between natural teeth and artificial teeth. Thus, it can be concluded that TrueTooth® serves as a suitable replica with potential application in endodontic training.

3. The described support material removal protocol can be concluded as effective in eliminating the support material from 3D printed teeth root canals.

4. The PolyJet™ technique is suitable for producing 3D printed teeth.

5. 3D printed teeth produced with High Temperature RGD525™ have similar behaviour to endodontic preparation that natural tooth, and 3D printed teeth can be used when comparing two preparation systems.

The potential exists for 3D printed teeth to establish a new standard in ex vivo studies and endodontic training, potentially supplanting natural teeth. However, achieving this requires further research to address the current limitations of 3D printed teeth. Additionally, there is a need for concerted efforts to establish robust protocols, facilitating method standardization and enabling comparison of results across studies.

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ABBREVIATIONS

3D – Three Dimensions

NiTi – Nickel-Titanium

nm – Nanometres

mm – Millimeters

mm^3 – Cubic Millimeters

º – Degrees

% – Percentage

Micro-CT – Micro Computed Tomography

µm – Micrometres

2D – Two Dimensions

CBCT – Cone Beam Computed Tomography

KV – Kilovolt

mA – Microampere

ms – milliseconds

Mbytes – Megabytes

ml – millilitres

MPa – MegaPascal

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1. INTRODUCTION

A section of this chapter has already been published as:

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1. INTRODUCTION

1.1 ENDODONTICS: DEFINITION AND OBJECTIVES

Endodontics includes the study and practice within dentistry focused on the structure, function, and diseases of the dental pulp and surrounding tissues. It delves into both fundamental and clinical sciences, exploring the biology of healthy pulp as well as the causes, diagnosis, prevention, and treatment of pulp-related injuries and associated conditions in the periradicular region (AAE, 2020).

Bacteria serve as the primary instigator of pulpal and periapical pathologies. Consequently, all endodontic procedures are directed towards eradicating both pulp tissue and as many bacteria as feasible (Liang et al., 2018; Ordinola-Zapata et al., 2014). According to the European Society of Endodontology, “the objectives of root canal system preparation are to: remove remaining pulp tissue, eliminate microorganisms, remove debris and shape the root canal system in order to be cleaned and filled” (ESE, 2006) .

The American Association of Endodontists defines root canal preparation as “Procedures involved in cleaning and shaping the canal system prior to obturation”, distinguishing between “biomechanical preparation” as the “use of rotary/reciprocating and/or hand files to expose, clean, enlarge and shape the pulp canal space, usually in conjunction with irrigants” and “chemomechanical preparation” as the “use of chemicals for irrigation of the root canal, demineralization of dentin, dissolution of pulp tissue and neutralization of bacterial products and toxins; used in conjunction with biomechanical preparation” (AAE, 2020).

The mechanical preparation of the root canal system stands as a pivotal stage in endodontics, as it dictates the effectiveness of all subsequent procedures that follow (Sinibaldi et al., 2012; Stavileci et al., 2013). The criteria for a well-prepared root canal involve preserving the original anatomy and the apical constriction, while ensuring a gradual tapering from the crown to the apex (Arias & Peters, 2022; ESE, 2006).

In pursuit of these objectives, a diverse array of manual rotary files has been introduced. The evolution in manufacturing techniques has resulted in a wide range of endodontic files, varying in design, alloys, and movement types (Alberton et al., 2020; Duque et al., 2017; Duque et al., 2019; Elashiry et al., 2020; Fernandes et al., 2020; Ordinola-Zapata et al., 2014).

Comprehensive understanding of the shaping characteristics and efficacy of rotary or reciprocating files is indispensable in aiding clinicians to choose the suitable system for diverse clinical scenarios. Given the continual introduction of new preparation systems into the market (Kirchhoff et al., 2015; Nagendrababu & Ahmed, 2019), clinicians rely on impartial evaluations of their features to inform their clinical decision-making process (Duque et al., 2017; Gagliardi et al., 2015; Pinheiro et al., 2018). Presently, there are over

200 NiTi preparation systems available in the market, yet the scientific validation of their clinical safety and efficiency remains largely unconfirmed (Aksoy et al., 2021; Arias & Peters, 2022).

Over recent decades, there have been notable shifts in root canal preparation protocols, with the emergence of numerous new nickel-titanium systems. These systems offer enhanced flexibility and resistance to torsion, leading to reduced working time, operator fatigue, and the likelihood of procedural mishaps. Nevertheless, clinicians still necessitate understanding the shaping properties and performance assessment of these systems to effectively match them with clinical cases (Nagendrababu & Ahmed, 2019; Pinheiro et al., 2018; Sousa-Neto et al., 2018).

Nickel-titanium (NiTi) files were initially introduced to the market in the 1990s, and since then, approximately 1280 studies have been published in PubMed-indexed journals. Previous bibliometric reviews have identified 'instrumentation' as the most frequently used term in titles and among the top-cited articles in endodontics. This collective information sheds light on root canal preparation emerging as the most cited keyword extracted from selected studies. These ex-vivo studies on root canal preparation offer valuable insights, aiding in the enhancement of biological outcomes. Hence, the continuation of such research efforts in the future remains essential (Aksoy et al., 2021; Arias & Peters, 2022; Hulsmann, 2022; Ordinola-Zapata et al., 2020; Yilmaz et al., 2019).

Key research areas in root canal preparation include dentin removal and volume expansion; straightening and transportation; unprepared root canal regions; and working time (Gancedo-Caravia et al., 2020; Hülsmann et al., 2005).

1.2 NiTi ALLOY

The NiTi alloy, coined "nitinol" from nickel (Ni), titanium (Ti), and Naval Ordnance Laboratory (NOL), originated from the United States space program and was formulated at the Naval Ordnance Laboratory in 1963. Renowned for its superelasticity, shape-memory effect, and biocompatibility, this alloy found application in orthodontic wire production as early as 1971 (Liang & Yue, 2022; Zupanc et al., 2018).

The equiatomic NiTi alloy consists of approximately equal proportions of nickel and titanium atoms (55% Ni and 45% Ti) and is characterized by three microstructural phases: austenite, martensite, and the R-phase. Austenite serves as the parent phase at elevated temperatures, while martensite acts as the daughter phase at lower temperatures, both constituting primary crystal structures of the NiTi alloy. Temperature and stress can induce alterations in the lattice crystal structure. Under normal conditions, the conventional NiTi alloy primarily adopts the austenitic form. However, within a narrow temperature range, a rhombohedrally distorted phase, known as the R-phase, may emerge, displaying a lower Young’s modulus compared to austenite and martensite. The R-phase NiTi alloy exhibits flexibility. Upon heating, martensite can transition back to austenite. In its austenitic state, the NiTi alloy demonstrates rigidity with shape memory properties, whereas in the martensitic phase, it becomes flexible, ductile, and easily deformable (Arias & Peters, 2022; Dablanca-Blanco et al., 2022; Hieawy et al., 2015; Liang & Yue, 2022; Peters et al., 2017; Tabassum et al., 2019; Thompson, 2000; Zanza et al., 2021; Zanza et al., 2022; Zupanc et al., 2018).

The phase composition and mechanical characteristics of the NiTi alloy are contingent upon the surrounding temperature and whether the alloy is subjected to cooling or heating processes. When the temperature surpasses the austenite finish temperature, the alloy assumes an austenitic state, exhibiting stiffness, hardness, and exceptional superelastic properties. Conversely, when the temperature falls below the martensite finish temperature, the NiTi alloy adopts a martensitic state, characterized by softness, ductility, ease of deformation, and the manifestation of the shape memory effect. Significant improvement in the mechanical properties of NiTi instruments have been attained through minor adjustments to the proportion of its constituent elements, however modifying the transformation temperatures of the NiTi alloy in use, which can be accomplished through thermal and mechanical treatments or adjustments in chemical composition, stands as the principal method for manufacturers to adjust the phase composition and, consequently, the mechanical characteristics of the NiTi alloy (Alqedairi et al., 2019; Arias & Peters, 2022; Dablanca-Blanco et al., 2022; Grande et al., 2017; Liang & Yue, 2022; Peters et al., 2017; Tabassum et al., 2019; Thompson, 2000; Zanza et al., 2022; Zupanc et al., 2018).

During clinical application, traditional NiTi alloy remains in the austenitic phase, whereas thermomechanical treated NiTi alloys exhibit varying proportions in the R-phase and martensitic phase. Such alterations result in endodontic files that are notably more flexible and possess enhanced fracture resistance. Over the years, numerous alloys have been developed through the heat treatment of NiTi (Arias & Peters, 2022; Thompson, 2000; Zanza et al., 2021).

In 2010, the market saw the introduction of NiTi control memory wire, featuring a reduced nickel content (52%) compared to conventional NiTi alloys (with nickel content ranging from 54.5% to 57%), leading to enhanced mechanical properties. Through post-manufacturing heat treatments, the crystal structure of these wires is primarily martensitic at room temperature. As a result, control memory wires have evolved into alloys with regulated memory, improved fracture resistance, excellent flexibility, and resistance to cyclic fatigue. These wires can be prebent to conform to the curvature of the root canal. If the instrument undergoes deformation, such as uncoiling, during use, it can revert to its original shape upon heating, as in an autoclave, allowing for reuse, a phenomenon known as the controlled shape memory effect. Blue and gold wires are obtained when the control memory wire alloy undergoes repeated heat and cooling treatments, forming a titanium oxide layer on its surface, with the surface colour varying based on the thickness of this layer. A thickness of 60–80 nm results in a blue surface colour, while a thickness of 100–140 nm yields a golden colour. This titanium oxide layer compensates for any hardness lost during the processing of the control memory wire alloy, thereby enhancing cutting efficiency and wear resistance (Arias & Peters, 2022; Dablanca-Blanco et al., 2022; Duque et al., 2019; Elnaghy & Elsaka, 2016; Liang & Yue, 2022; Tabassum et al., 2019; Zupanc et al., 2018).

1.3 ENDODONTIC FILES DESIGN

NiTi rotary files appeared by 1990s, and one of the major advancements was the change related to file taper, compared with ISO-normed hand files with 0.02 taper. NiTi alloy flexibility made possible the increase of taper and still prepare curved canals in a safe manner. In contrast with stainless steel that follow a unified international standard, for the design of rotary NiTi files it has not been established. In this way, the geometric

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design of rotary NiTi files has undergone continuous refinement to achieve an equilibrium between cutting effectiveness and safety. Endodontic companies persist in developing files that are both more efficient and safer. This advancement is achieved either through alterations in the mechanical properties of the NiTi alloy, as mentioned earlier, or through modifications in file design, encompassing aspects such as tip and taper design, the presence of radial lands, rake angles, helical angles, and pitch, as well as the type of movement kinematics (Alqedairi et al., 2019; Arias & Peters, 2022; Dablanca-Blanco et al., 2022; Grande et al., 2005; Liang & Yue, 2022; Sanghvi & Mistry, 2011; Silva et al., 2023).

The tip of a file can be categorized into two types: cutting and non-cutting tips. The design of the tip significantly influences the file's ability to navigate obstacles within the canal and its likelihood of causing clinical damage. A file with a cutting tip is more effective in narrow canals due to its aggressive nature but poses greater risk when used in curved canals compared to a non-cutting tip (Grande et al., 2005; Peters et al., 2017; Sanghvi & Mistry, 2011).

The taper refers to the ratio of an instrument's diameter to its length, representing the rate of diameter increase per millimeter along its working surface, from the tip towards the file handle. For instance, a 30-tip file with a taper of 0.2 would exhibit a diameter of 0.32 mm at 1 mm from the tip and 0.34 mm at 2 mm from the tip and so on. The taper can be constant or variable. There are two options for preparing a canal. Firstly, using files with a constant taper but with different tip sizes. Secondly, employing files with variable tapers on the same file or graduating tapers while maintaining the tip size. The idea behind the latter choice is that each subsequent file engages only a small portion of the canal wall. As a result, frictional resistance is reduced, leading to lower torque requirements for operation (Dablanca-Blanco et al., 2022; Liang & Yue, 2022; Sanghvi & Mistry, 2011).

The radial land is a surface located directly behind the cutting edge. It aids the instrument's central movement within the root canal without inducing canal transportation. A broader radial land enhances the peripheral strength of the instrument, albeit at the cost of heightened friction during dentin cutting, requiring additional torque and time for effective operation during work (Dablanca-Blanco et al., 2022; Liang & Yue, 2022; Sanghvi & Mistry, 2011).

The rake angle relates to the inclination and placement of the cutting edge on the cross-section in relation to the dentin surface and is defined as the angle created by the cutting edge and a cross-section taken perpendicular to the long axis of the instrument. The cutting angle can be either positive or negative. A positive rake angle design enables high cutting efficiency but results in rapid wear. An excessively positive rake angle may cause digging and gouging of the dentin, potentially resulting in instrument separation. Conversely, instruments with a neutral or negative rake angle primarily grind against the root canal wall, focusing more on scrapping than cutting, thus diminishing dentin cutting efficiency (Dablanca-Blanco et al., 2022; Grande et al., 2005; Ha et al., 2017; Liang & Yue, 2022; Sanghvi & Mistry, 2011).

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The helical angle refers to the angle formed between the cutting edge and the long axis of the file. As a rotary file operates within the canal, it is crucial to effectively remove dentinal debris. Files with a constant helical angle may accumulate debris and are more prone to screw in forces or the sensation of being pulled into the canal as the file rotates.

Varying the helical angle facilitates more efficient debris removal and reduces the likelihood of the file screwing into the canal, thereby decreasing the torsion effect on the file (Dablanca-Blanco et al., 2022; Ha et al., 2017; Liang & Yue, 2022; Sanghvi & Mistry, 2011).

The pitch indicates the quantity of spirals per unit of length. A smaller pitch implies more spirals on the file. When both the pitch and helical angles remain constant, this results in higher "screw-in" tendency (Dablanca-Blanco et al., 2022; Sanghvi & Mistry, 2011).

Continuous rotary motion enables the file to cut the root canal wall continuously through full rotation. Reciprocating motion was introduced by Yared in 2008, suggesting the use of engine-driven NiTi systems employing a balanced-force technique. This involves preparing the root canal through alternating clockwise and counterclockwise rotations at different angles. In most reciprocating systems, the file rotates approximately 120°–270° in the counterclockwise direction to cut dentin, followed by clockwise rotation spanning 60°–90° to remove debris. This alternating motion helps alleviate torsional stress (Dablanca-Blanco et al., 2022; Liang & Yue, 2022).

It is advisable to utilize repeated pecking motions, also known as repetitive in-and-out movements, when shaping root canals with NiTi rotary instruments. Such motions are particularly effective for shaping the apical portion of the canal space. Pecking is essential for dispersing the flexural stresses generated during the rotation of instruments in curved canals, thereby reducing the risk of separation. Shorter movements help minimize screw-in forces, thereby reducing levels of torsional stress by providing a pause for the instruments within the highest stress area. By employing pecking judiciously, root canal instrumentation can achieve better preparation quality (Ha et al., 2017; Ha & Park, 2012; Kimura et al., 2020; Kyaw et al., 2021; Wang et al., 2022).

The initial step in preparing the root canal system involves establishing a glyde path. This is achieved by employing stainless steel hand files or NiTi rotary instruments with a small diameter and taper. Creating a glyde path entail crafting a smooth pathway from the root canal orifice to the apical foramen. This crucial procedure enhances the efficacy and safety of subsequent cleaning and shaping processes within the root canal system. Notably, mechanically glyde path formation diminishes torsional stresses, prolongs the lifespan of the files, and reduces the occurrence of procedural errors during root canal preparation, as also has been associated with shorter operation times (Alovisi et al., 2017; Keskin et al., 2020).

Various mechanical files are available on the market for creating a glide path. The ProGlyder® file (Dentsply Sirona) features a 0.16 mm tip size and a variable taper ranging between 2% and 8.5% (Alovisi et al., 2017; Gambarini et al., 2021; Keskin et al., 2020; Kirchhoff et al., 2015; Paleker & van der Vyver, 2017). The A file, part of the Endogal® system (Endogal Galician Endodontics Company), presents a uniform 3% taper with a tip size of 0.15 mm (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021).

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1.3.1 ProTaper Gold® system

The ProTaper Gold® (Dentsply-Sirona) maintains an identical sequence of six instruments and shares the same design and geometric attributes as the ProTaper

Universal® (Dentsply-Sirona) system except that uses gold wire. The standard ProTaper set consists of six files: three Shaping Files (SX, S1, S2) and three Finishing Files (F1, F2, F3). The SX file, begins with a diameter of 0.19 mm at D0, with its taper gradually increasing from the tip to the handle. This file is employed to achieve wider flaring in the coronal portion of long roots. Additionally, the SX file can serve as an alternative to Gates Glidden burs for enlarging the preparation. It is recommended to use lateral pressing movements on dentin walls (brushing). The diameter of the S1 Shaping File at D0 is 0.18 mm and is designed to prepare the coronal third of the canal, while the diameter of the S2 Shaping File is 0.2 mm that enlarges and prepares the middle third while simultaneously providing access to the apical third. The diameters F1, F2, and F3, at D0 size are 0.2 mm, 0.25 mm, and 0.3 mm, respectively and are intended to complete the apical third, they exhibit a progressive taper. For F1, the taper initiates at 7%; for F2 at 8%; and for F3 at 9%. The taper remains constant for the first 3 mm, allowing flexibility in selecting the cone's taper for canal filling. The ProTaper Gold® (Dentsply-Sirona) features a triangular convex shape in its transverse section, with three cutting surfaces. These files exhibit a varying helical angle and pitch. The rake angle of these instruments is negative, and their tips are rounded and non-cutting (Aazzouzi-Raiss et al., 2023; Alqedairi et al., 2019; Duque et al., 2017; Duque et al., 2019; Gagliardi et al., 2015; Grande et al., 2005; Nehme et al., 2021; Pinheiro et al., 2018; Silva et al., 2023; Silva et al., 2021; Yalniz et al., 2021).

1.3.2 Endogal® system

The Endogal® system (Endogal Galician Endodontics Company) introduces a novel approach utilizing Gold Wire with a parallelogram cross-section. It features files with a consistent 4% taper, available in tip sizes of 0.2 mm (File B) and 0.25 mm (File C). Additionally, it offers files with a 6% taper, ranging from tip sizes of 0.25 mm (File D) to 0.50 mm (File G). Furthermore, the system includes an X file specifically designed for removing coronal interferences, featuring a tip size of 0.25 mm and a 9% taper. These files can be utilized in either continuous or reciprocating movement (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021).

1.4 MICRO COMPUTED TOMOGRAPHY

Originating in the early 1970s, Computed Tomography imaging has greatly influenced clinical diagnostic imaging. Computer Tomography images, reconstructed from multiple viewing angles, offer a significant advantage over conventional radiography images by providing quantitative information about the spatial distribution of material density within an object. In contrast, conventional radiography only provides a sum of material attenuation along the X-ray path. Clinical Computed Tomography scanners typically generate images composed of 1 mm^3 volume elements (voxels), while Micro Computed Tomography (Micro-CT) systems, developed in the early 1980s, offer voxel spacing ranging from 5 to 50 μm (du Plessis & Broeckhoven, 2019; Ghavami-Lahiji et al., 2021; Guldberg et al., 2003; Muller, 2009; Stauber & Muller, 2008).

In recent years, Micro-CT has evolved from being solely a qualitative imaging tool to becoming a quantitative analytical tool, offering significant advantages across various fields. This transition has been thoroughly examined in materials sciences, biological sciences, industrial applications, and additive manufacturing (du Plessis & Broeckhoven, 2019).

Presently, Micro-CT technology is acknowledged as the foremost and precise research tool for examining the root canal system. Its significance extends to comprehending the impact of its intricate morphology on various phases of endodontic treatment, among other applications. In odontogenesis studies, the most reported results are comparative volumetric measurements, densitometric data, and, less frequently, morphometric analyses (Aksoy et al., 2021; Verdelis & Salmon, 2019).

Micro-CT is known for its non-invasive and non-destructive nature, offers high-resolution imaging capabilities. Because the imaging process is non-destructive, it allows for multiple examinations of the internal features of the same sample. Additionally, samples remain intact and available for further biological and mechanical testing after scanning. It enables the comprehensive three-dimensional exploration of the root canal system, providing valuable insights into its influence on various treatment and retreatment procedures. By digitally reconstructing cross-sectional images of teeth, micro-CT facilitates the creation of 3D volumes. These volumes can then be utilized to generate computerized representations of specimens, allowing for manipulation and precise measurements of both internal and external morphologies (du Plessis & Broeckhoven, 2019; Ghavami-Lahiji et al., 2021; Guldberg et al., 2003; Versiani, Leoni, et al., 2013; Versiani et al., 2012; Versiani, Pecora, et al., 2013).

The micro-CT procedure comprises several steps, including, sample preparation and mounting; configuring the scanner and selecting parameters; conducting the scanning process; reconstructing the images, and visualizing the obtained images (du Plessis et al., 2017).

The Micro-CT setup consists of several essential components: an X-ray source, a sample undergoing rotation, and a digital detector. The X-ray source emits a cone beam of X-rays, which pass through and around the object being examined, casting a shadow image on an X-ray detector. This digital detector captures 2D X-ray projection images at each step position during a complete rotation of the sample around an axis perpendicular to the X-ray beam direction. These X-ray absorption images, also called projection images, provide various views of the sample from different angles, revealing internal details due to X-ray penetration. Afterward, the acquired images undergo reconstruction using a filtered back projection algorithm, resulting in a 3D dataset containing volumetric pixels, known as voxels. The brightness of each voxel reflects the density and atomic mass of the corresponding material (du Plessis & Broeckhoven, 2019; du Plessis et al., 2017; Du Plessis et al., 2018; Ghavami-Lahiji et al., 2021; Guldberg et al., 2003; Muller, 2009; Stauber & Muller, 2008; Verdelis & Salmon, 2019).
In addition to its non-invasive and non-destructive nature, this method offers several other advantages in dental research. 3D analysis facilitates measurements across the entire volume of interest, providing statistically more reliable data compared to traditional 2D analysis modes, which often focus on sections or the surface of a specimen. Furthermore, the ability to digitally reorient imaged volumes allows for optimal viewing and processing, enhancing the versatility and accuracy of analysis (Verdelis & Salmon, 2019).

However, Micro-CT has several limitations. Its restricted field of view confines studies to small samples, usually limited to extracted teeth. Additionally, it is time-consuming, requiring several hours for the acquisition and reconstruction of a single tooth image. Furthermore, it involves higher irradiation doses compared to conventional medical scanners. As a result, it is exclusively reserved for in vitro endodontic research studies

(Ghavami-Lahiji et al., 2021; Michetti et al., 2017; Orhan & Büyüksungur, 2020; Queiroz, Rovaris, Gaeta-Araujo, et al., 2017).

1.4.1 Micro-CT procedure

1.4.1.1 Sample preparation

Micro-CT necessitates minimal sample preparation, often allowing scanning of the sample in its original state. However, due to the rotating sample configuration proper sample loading is crucial to prevent movement during scanning. Sample mounting involves utilizing lightweight materials to securely hold the sample in place while ensuring separation from the dense hardware of the rotation stage, with their long axis aligned with that of the holder (the scan's z-axis) to minimize beam hardening (du Plessis et al., 2017; Verdelis & Salmon, 2019).

1.4.1.2 Establishing scan parameters

When configuring the scan, it is important to choose the voxel size, ideally set to three times smaller than the thickness of the thinnest part of the object. Voxel size plays a crucial role in determining image quality. Therefore, variations in image quality could influence the efficacy of segmentation techniques, subsequently impacting both quantitative and qualitative measurements. Selecting the X-ray beam energy involves determining the combination of the X-ray filter and applied voltage. Most systems offer customization of a scanning protocol, allowing users to set parameters such as exposure time, which dictates the duration of continuous irradiation for the detector at each lateral view, the frame averaging per view (the number of repeated lateral views acquired at a specific rotation step), and the number of rotation steps during a scan (typically 180 or 360 degrees). It's essential to consider that higher resolution scans necessitate longer acquisition times and result in larger data sets (Orhan & Büyüksungur, 2020; Orhan et al., 2018; Torres et al., 2022; Verdelis & Salmon, 2019).

Optimization of scan parameters is essential for each sample type, typically involving the selection of lower voltages for biological materials to improve contrast. Denser objects may necessitate higher voltages for increased penetration capability, although this can diminish the contrast between materials with similar densities. Enhancing the quality of results involves acquiring a greater number of images during rotation. Subsequently, reconstruction occurs with specific parameters tailored to the system (du Plessis & Broeckhoven, 2019; du Plessis et al., 2017; Du Plessis et al., 2018; Stauber & Muller, 2008).

1.4.1.3 Scanning procedure

After loading the sample and selecting the settings, image acquisition can commence. The scanning process operates automatically without requiring user intervention (du Plessis et al., 2017).

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1.4.1.4 3D reconstruction of the scan

Once all 2D image projections have been obtained, a 3D volume can be generated. The 3D image is generated from a stack of 2D slices through reconstruction. The spacing between two slices is determined by the pixel size utilized during the scanning process. The reconstruction process entails mapping each voxel using projection image representations captured from various angles. This mapping is accomplished through a Feldkamp filtered back-projection algorithm. The reconstruction software comprises a range of settings that could impact the quality of the resulting 3D data. Depending on the system, users may need to adjust certain reconstruction parameters, such as beam hardening and ring artifact correction, or specify a range of attenuation coefficient values to define the intensity window for grayscale values in the reconstructed volume (du Plessis et al., 2017; Orhan & Büyüksungur, 2020; Verdelis & Salmon, 2019).

Artifacts are discrepancies in image reconstruction that do not belong to the scanned object. However, it is possible to minimize (or eliminate) artifacts by carefully selecting scanning parameters and/or employing artifact reduction tools during the image reconstruction process. Various types of artifacts can occur in micro-CT imaging. Beam hardening occurs when low-energy photons are absorbed more than higher-energy photons within the beam, leading to an increase in average photon energy along the beam path due to the depletion of low-energy photons. This results in the beam becoming "harder" or more penetrating after passing through the object, causing various artifacts in the reconstructed image, such as the cupping artifact or lighter areas around the edges of the material, as well as streaks or hypodense bands. The cupping artifact arises from underestimating the true density of the object. Another common artifact is the ring artifact, characterized by concentric rings in the image, often due to detector damage or calibration issues and the circular scanning geometry. The presence of artifacts can compromise the analysis of micro-CT images and, consequently, affect the outcomes of the study being examined (du Plessis et al., 2017; Orhan & Büyüksungur, 2020; Orhan et al., 2020; Queiroz, Rovaris, Gaeta-Araujo, et al., 2017; Stauber & Muller, 2008; Verdelis & Salmon, 2019).

1.4.1.5 Processing reconstructed volume

The collected data is subsequently analysed and visualized utilizing specialized software, which allows for material segmentation in the slice images and facilitates 3D visualization and additional processing (du Plessis & Broeckhoven, 2019; Du Plessis et al., 2018; Stauber & Muller, 2008).

To determine the volume and surface area of the root canal, the process commences with image segmentation. Initially, thresholding is applied to the images, resulting in binarization (black and white representation). It's imperative to precisely establish the grey value thresholds for dental hard tissues and root canal spaces, as inaccuracies in this step may lead to over- or underestimation of measurements. The accurate inclusion of the region of interest in segmentation relies on the careful selection of threshold values. These values dictate whether an area is deemed white and included in the analysis or black and excluded from it (Michetti et al., 2017; Queiroz, Rovaris, Santaella, et al., 2017).

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Micro-CT technology enables the generation of color-coded 3D models of root canals by integrating co-registered datasets from pre- and post-preparation stages with high-precision image registration software. A micro-CT scan provides comprehensive information; slices can be reconstructed in any plane, and data can be presented as either 2D or rendered 3D images. Internal and external anatomy can be examined simultaneously or separately. Additionally, images can be assessed both qualitatively and quantitatively. Through this method, analyses of root canal morphology, cracks, and the efficacy of various systems in shaping, cleaning, and filling have been conducted (Ghavami-Lahiji et al., 2021; Verdelis & Salmon, 2019; Versiani & Keleș, 2020; Versiani, Leoni, et al., 2013; Versiani et al., 2012; Versiani, Pecora, et al., 2013).

Endodontic preparation involves the abrasion of dentin, resulting in changes to the geometric shape of the root canal. Micro-CT systems have simplified and streamlined the three-dimensional evaluation of canal preparation. Utilizing data obtained from these scans, it becomes possible to quantify numerous changes occurring before and after preparation. These changes encompass alterations in the surface area and volume of the root canal, the volume of dentin removed, unprepared areas, canal transportation, structure model index, and transportation of centres of mass. Typically, researchers establish the pre-operative micro-CT image as a reference and align the post-operative image until an optimal fit is attained. This process, termed image registration or superimposition, involves spatially overlaying 3D images. By merging and visualizing pre- and post-operative canal morphology through image registration, researchers attain a comprehensive understanding of the instrumentation outcome (Drukteinis et al., 2019; Lui et al., 2023; Swain & Xue, 2009).

1.5 THREE- DIMENSIONAL PRINTED TEETH

1.5.1. Why 3D printed teeth are needed for endodontics's science and teaching community

In ex vivo studies and pre-clinical courses, natural teeth remain the preferred choice (Bitter et al., 2016; De-Deus et al., 2020; Decurcio et al., 2019; Dobros et al., 2022; Hulsmann, 2022; Reymus et al., 2019). Their benefits encompass natural tissue hardness, morphology, colour, texture, and radiodensity (Decurcio et al., 2019; Dobros et al., 2022). However, as previously mentioned, they are accompanied by several limitations.

Challenging to collect, gathering an ample supply of suitable natural teeth has become increasingly difficult, likely due to advancements in health standards. This process is time-intensive and demands a substantial quantity of teeth. Wisdom teeth, teeth with highly intricate root canal morphology, and those with prior root canal treatment are unsuitable. Additionally, natural teeth must exhibit complete root development, an intact crown, and undamaged roots (Al-Sudani & Basudan, 2017; Bitter et al., 2016; Decurcio et al., 2019; Gancedo-Caravia et al., 2020; Hulsmann, 2022; Tchorz et al., 2015; Yekta-Michael et al., 2021). Nowadays, biobanks stand as fundamental resources for advancing precision medicine. Biobanks are designed to bolster and enhance clinical and biomedical research, particularly for preclinical trials (Curylofo-Zotti et al., 2018; Sivolella et al., 2022). Consequently, natural teeth from such biobanks are unavailable for educational use.

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Ethical concerns arise regarding the previous owners of natural teeth, as their consent for the use of their teeth as biomaterial should have been obtained (Dobros et al., 2022; Holden & Dracopoulos, 2017; Hulsmann, 2022). A troubling trend is the purchase of human teeth online, where pressures to acquire teeth may lead investigators and students to overlook inquiries into the origins and reasons behind the extraction of these teeth (Holden & Dracopoulos, 2017).

Potential risks of cross-infection and storage: natural teeth are heavily contaminated, posing challenges for sterilization. Common disinfection methods like sodium hypochlorite and hydrogen peroxide are often ineffective (Hope et al., 2013; Kumar et al., 2005). Furthermore, sterilization and storage procedures may damage or alter teeth. Research indicates that autoclaving reduces teeth's resistance to fracture (Michaud et al., 2018). Similarly, the storage conditions affect tooth integrity, with teeth stored in water exhibiting higher resistance to fracture than those stored dry (Mello et al., 2020). Consequently, preparing natural teeth entails unpleasant tasks such as storing them in strong-smelling liquids (Kroger et al., 2017).

Standardization poses a challenge due to the complex anatomy of the root canal system, resulting in difficulties in establishing well-balanced study groups and ensuring fair student evaluations (De-Deus, 2012; De-Deus et al., 2020; Orel et al., 2021; Reymus et al., 2019; Reymus et al., 2020; Versiani, Pecora, et al., 2013). Consequently, studies may reflect the impact of canal anatomy rather than the specific variable under investigation (De-Deus, 2012).

Students often express concerns that anatomical variations hinder the validity of individual performance assessments (Tchorz et al., 2015).

In ex vivo studies, the prevalent approach involves pairing tooth samples based on shared anatomical characteristics, including tooth type, root length, root anatomy, curvature degree and angle, and root canal diameter. In some cases, teeth are paired with contralateral teeth. Randomization methods, with or without the aid of radiographs, are also utilized to allocate teeth from the same group (De-Deus et al., 2020; Hulsmann, 2022; Johnsen et al., 2017; Johnsen et al., 2018; Xu et al., 2016).

Recently, certain studies have begun utilizing specific anatomical parameters identified through micro-CT scanning (De-Deus et al., 2020; Hulsmann, 2022). De-Deus et al. (2020) conducted a comparison between the randomized method, radiographic method, and micro-CT method. The findings indicated that the micro-CT method offered superior control over confounding anatomical variances. However, this study commenced with a pool of 1708 single-rooted mandibular incisors. From this pool, 251 teeth were selected for the micro-CT group, which underwent scanning to establish two anatomically paired groups (n=10) (De-Deus et al., 2020). This highlights the challenges, time consumption, and high costs associated with this method.

Furthermore, it's important to recognize that certain tooth parameters, including donor age, ethnicity, and environmental factors, cannot be adequately standardized through these methods. These factors influence the mechanical properties of dentin (Hulsmann, 2022; Ivancik et al., 2014; Panfilov et al., 2016). Dentin, a hard tissue occupying the bulk of the human tooth, boasts a complex hierarchical structure. It

comprises around 45% mineral material, 33% organic material, and 22% water (Montoya et al., 2015; Panfilov et al., 2016).

The microstructure of dentin is primarily defined by its tubules, which radiate outward from the pulp toward the dentin–enamel junction. Each tubule contains a lumen encased by peritubular dentin, comprising densely mineralized apatite crystals and a small portion of organic proteins. Intertubular dentin, situated between the tubules, is characterized by a matrix of collagen fibers fortified by apatite (Montoya et al., 2015; Ryou et al., 2015).

Following the third decade of life, a shift occurs in the microstructure of dentin, marked by an increase in inorganic material content as the tubules gradually fill with it (Montoya et al., 2015; Montoya et al., 2016; Panfilov et al., 2016; Ryou et al., 2015). This leads to a reduction in tubule density and dentinal diameter. These changes result in alterations to its mechanical properties, including heightened elastic modulus and hardness, alongside diminished strength and fatigue crack resistance (Li et al., 2015; Montoya et al., 2015; Montoya et al., 2016; Pashley, 1985; Ryou et al., 2015). The aging process of dentin commences from the root dentin and progresses towards the coronal direction (Montoya et al., 2015; Yan et al., 2017). In older donors, the occlusion rate of dentinal tubules is highest at the root apex and closer to the pulp, while in younger donors, it is greater near the cementum (Yan et al., 2017). In this manner, the age of the donors exerts an influence on the properties of the dentin. In this way, the use of extracted teeth obtained from patients of unknown age may have the potential to significantly impact the results (Alak et al., 2023; Arola et al., 2017; De-Deus et al., 2023). Moreover, a study examining the microstructure and mechanical properties of dentin revealed disparities between donors from the United States of America and those from Colombia (Ivancik et al., 2014).

Due to the aforementioned reasons, achieving complete standardization with current methods is nearly impossible.

3D-printed teeth, which emulate the characteristics of natural teeth, have the potential to address their constraints (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Reymus et al., 2021; Reymus et al., 2020). Moreover, they offer a practical way for developing the skills in access opening, canal preparation, radiographic length control, and canal filling (Dobros et al., 2022; Reymus et al., 2019).

Challenging to gather: There are no issues with collection, as the selection of various teeth is unrestricted and they are readily available in abundant quantities (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Hulsmann, 2022; Kroger et al., 2017; Reymus et al., 2019). Furthermore, an online platform facilitating institutions to exchange their printable files would greatly benefit the community as a whole (Reymus et al., 2019).

Ethical concerns: Only 3D printed teeth created from cone beam computer tomography (CBCT) or micro-CT scans of actual teeth should necessitate donor consent for their use (Dobros et al., 2022; Holden & Dracopoulos, 2017; Hulsmann, 2022); nevertheless, the quantity of natural teeth required would be minimal.

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Potential for cross-infection and storage concerns: they pose no risk of cross-infection, offer enhanced hygiene, and are easier to handle as they do not require storage

in liquid, ensuring a secure training environment (Al-Sudani & Basudan, 2017; Kolling et al., 2021; Kroger et al., 2017).

Standardization: they are realistic and standardized, ensuring consistent difficulty levels for every student. Consequently, fair scoring is guaranteed (Al-Sudani & Basudan, 2017; Bitter et al., 2016; Dobros et al., 2022; Fakhr & Nagy, 2023; Kroger et al., 2017; Pouhaer et al., 2022; Reymus et al., 2019), facilitated by the establishment and standardization of specific grading criteria (Gancedo-Caravia et al., 2020; Lugassy et al., 2021). This standardization enables students and instructors to concentrate on learning and teaching clinical procedures without being hindered by the morphological diversity of natural teeth (Decurcio et al., 2019). Furthermore, in ex vivo studies, this morphological standardization significantly enhances result credibility (De-Deus, 2012; De-Deus et al., 2020; Karatekin et al., 2019; Ordinola-Zapata et al., 2014; Xu et al., 2021; Zhu et al., 2024).

3D printed teeth offer additional advantages in pre-clinical courses by gradually introducing canal anatomy complexities (Al-Sudani & Basudan, 2017; Fakhr & Nagy, 2023; Ordinola-Zapata et al., 2014). Students have the opportunity to practice procedures repeatedly and compare various protocols, a benefit also applicable to ex vivo studies (Al-Sudani & Basudan, 2017; Fakhr & Nagy, 2023; Ordinola-Zapata et al., 2014; Yekta-Michael et al., 2021). It follows logically that techniques thoroughly covered in pre-clinical courses are executed more confidently and with less stress when students treat patients for the first time (Hanafi et al., 2020).

In a study by Tchorz et al. (2015), the impact of students training with artificial teeth versus extracted human teeth on the quality of their initial root canal treatments performed on patients was compared. They found no discernible differences between the two groups (Tchorz et al., 2015), indicating that artificial teeth are indeed appropriate for endodontic training.

Several studies highlight students' strong positive feedback regarding the utilization of 3D printed teeth for standardized endodontic training and as realistic simulations of clinical treatment (Kolling et al., 2021; Reymus et al., 2019; Reymus et al., 2021). Furthermore, instructors also value 3D printed teeth, likely due to their firsthand experience with the challenges faced during pre-clinical courses using natural teeth (Bitter et al., 2016; Gancedo-Caravia et al., 2020).

Another application of 3D printed teeth is in addressing complex anatomical clinical cases. This enables clinicians to comprehend the root canal configuration and test specific clinical treatments before implementing them on a patient. By training with 3D printed models, clinicians can explore various approaches, enhancing their confidence levels for the actual treatment procedure (Byun et al., 2015; Liang et al., 2018; Tonini et al., 2021).

1.5.2 How 3D printed teeth are manufactured

Additive manufacturing, commonly referred to as 3D printing, involves the fabrication of parts by merging materials based on 3D model data, typically through layer-by-layer deposition (ISO/ASTM, 2021). This innovative technology utilizes 3D data, post-processing tools, and algorithms to manipulate and adjust multiple layers, resulting in the creation of a 3D model. Subsequently, a 3D printer employs a specific material to

produce the desired part (Liang et al., 2018; Shah & Chong, 2018; Xu et al., 2021) (Figure 1).

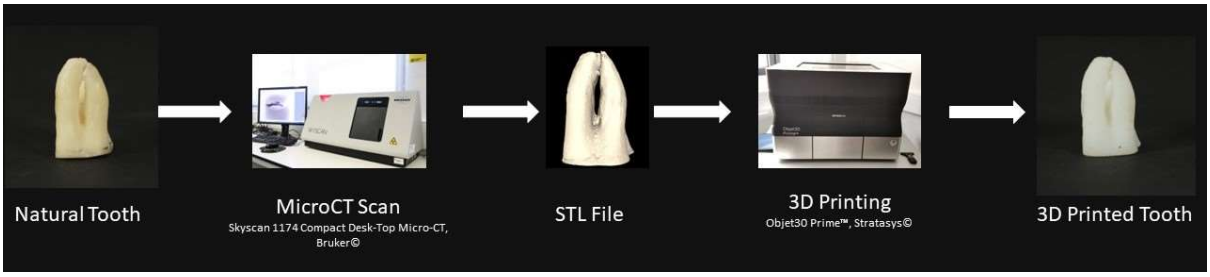

Figure 1. 3D printing process (https://www.mdpi.com/about/openaccess)

The initial phase of 3D printing teeth involves acquiring a digital model derived from computer tomography scan data, laser scan data, or created through computer-aided design (Shah & Chong, 2018; Walker & Humphries, 2019). The scan data undergo processing through 3D construction software to produce data in STL format (Cui et al., 2018; Xu et al., 2021).

The STL format, which stands for Standard Tessellation Language or Standard Triangulation Language, represents virtual 3D surfaces using triangulation (tessellation). Each triangular face is defined by three corner points and the corresponding surface. Curved surfaces are approximated by polygons. Increasing the number of polygons, by reducing the size of the triangles, enhances the object's surface resolution. However, this also significantly increases the number of triangles in the file, leading to larger file sizes (Kessler et al., 2020; Paul & Anand, 2015).

The precision of 3D printers is determined by two main factors: XY resolution, which relates to horizontal accuracy, and layer thickness, representing the Z-axis or vertical accuracy. Layer thickness generally dictates the surface quality, with thinner layers yielding smoother objects. However, thinner layers also result in longer printing times (Anderson et al., 2018; Kessler et al., 2020).

In certain 3D printing methods, support material is utilized to fill areas beneath overhangs, within cavities, and holes, and the process of removing this support material varies depending on the technology employed (Kessler et al., 2020).

Stereolithography (layer thickness 25–100 μm) The polymerization process involves a single ultraviolet laser and a vat containing liquid resin (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016; Reymus et al., 2020; Walker & Humphries, 2019). A computer-controlled mirror directs the ultraviolet laser onto the resin surface, curing it layer by layer. Through sequential curing, the layers fuse together to form a solid mass, starting from the bottom of the object and progressing upwards; this method is known as the bottom-up approach (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016). Alternatively, in the top-down approach, the platform is lowered vertically into the reservoir, and the resin layer is exposed to a laser scanning from the bottom of the reservoir (Kessler et al., 2020). Following printing, the model is removed and undergoes additional post-curing in a ultraviolet cabinet to reduce residual resin content (Kessler et al., 2020; Kim et al., 2016). The resulting model material is robust, slightly brittle, and

relatively lightweight (Kim et al., 2016). Support material is necessary for this process (Kessler et al., 2020).

Digital Light Processing (layer thickness 25–100 µm) The polymerization occurs through a light source emitted from a traditional Digital Light Processing projector, with the liquid resin contained within a vat (Kessler et al., 2020; Kim et al., 2016; Reymus et al., 2020). The projector illuminates the resin with a 2D image, building the object layer by layer as the platform undergoes manipulation (Anderson et al., 2018). This process resembles Stereolithography, albeit without a moving beam (Shah & Chong, 2018). Support material is necessary for this process (Kessler et al., 2020).

Fused Deposition Modelling, with layer thickness options of 178 or 254 µm, involves depositing layers of molten material from a filamentous nozzle which then solidify within 0.1s. Typically, Fused Deposition Modelling is characterized by lower accuracy (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016; Lee et al., 2015; Walker & Humphries, 2019), and may require additional support material (Kessler et al., 2020).

ColorJet printing technology (with a layer thickness of 50–100 µm) operates by selectively dispersing a binding agent onto layers of powder, which are then fused together (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016; Walker & Humphries, 2019). Nevertheless, its accuracy, surface finish, and strength are generally poor (Shah & Chong, 2018). Unlike other methods, support material is unnecessary because the surrounding powder provides support for unconnected parts. During the finishing process, a cyanoacrylate-based material is infused into the prototype to reinforce its structure and reduce porosity (Kessler et al., 2020; Kim et al., 2016).

Selective Laser Sintering printers (with a layer thickness of 30–100 µm) and Selective Laser Melting printers (with a layer thickness of 20–100 µm) employ a computer-guided laser and a roller mechanism to distribute layers of powdered material onto the preceding layer. Subsequently, each new layer undergoes sintering (Selective Laser Sintering) or melting (Selective Laser Melting) (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016; Walker & Humphries, 2019), allowing for the printing of thermoplastic polymer, metal, ceramic, or glass (Kessler et al., 2020; Shah & Chong, 2018). These printers operate without the need for support material (Kessler et al., 2020).

PolyJet™ and MultiJet printing technology, with layer thickness capabilities of up to 16 µm, employs photosensitive resin to achieve exceptional precision in product detailing. This method transcends limitations imposed by geometric shapes and is particularly adept at simulating root canal anatomy. However, its limitations include the device's high cost and the instability of resin-based materials over time, which is a common issue with all resin-based technologies (Rouze l'Alzit et al., 2022). Conceptually comparable to the 2D printing process of an inkjet printer, it offers remarkable accuracy (Kessler et al., 2020; Kim et al., 2016; Kolling et al., 2021; Kroger et al., 2017; Liang et al., 2018; Walker & Humphries, 2019). This technology enables the printing of two distinct materials within a single print job: a model material and a supporting material (Kessler et al., 2020; Kim et al., 2016; Kroger et al., 2017; Lee et al., 2015; Shah & Chong, 2018). The process involves the printer nozzles moving along the XY plane, dispensing liquid photosensitive resin onto the printer bed, followed by curing the resin with a ultraviolet lamp. Once the initial layer is complete, the printer bed lowers by the specified layer thickness in the Z plane, and the deposition of subsequent layers repeats. This

method holds promise as an appealing choice for low-volume manufacturing within research environments. In areas where hollow parts or overhangs are present, the nozzles apply a layer of removable support material (Anderson et al., 2018; Fahad et al., 2013; Herpel et al., 2021; Kent et al., 2017; Kim et al., 2016; Kroger et al., 2017; Lee et al., 2015; Macdonald et al., 2017; Quan et al., 2020; Walker & Humphries, 2019). Methods for removing support material include manual breaking, dissolution under water pressure or with sodium hydroxide, or melting (Fahad et al., 2013; Kent et al., 2017; Kessler et al., 2020; Kroger et al., 2017; Stratasys, 2019). The distinction between these two printing technologies lies solely in their support materials and post-processing demands (Anderson et al., 2018; Kessler et al., 2020; Kim et al., 2016).

1.5.3 Problems with 3D printed teeth

3D printed teeth are currently limited to studies focusing solely on root canal shape and are not suitable for assessing root canal cleanliness or dentin alterations (Hulsmann, 2022). Nevertheless, recent investigations by Choi et al. (2021) and Teves Cordova et al. (2024) have proposed enhancements to a formulated 3D printed tooth by integrating a slot for inserting a dentin segment. This adaptation allowed the evaluation of different irrigation protocols for both bacterial contamination of the dentin and smear layer removal, respectively. However, additional research utilizing this prototype is necessary (Choi et al., 2021; Teves Cordova et al., 2024).

However, they hold potential for use in studies concerning obturation. Karatekin et al. (2019) and Gok et al. (2017) assessed various obturation techniques in 3D printed teeth featuring C-shaped canals, employing cross-sectional analysis at different levels, and examining images under magnification (Gok et al., 2017; Karatekin et al., 2019). In Karatekin et al.'s study, 3D printed teeth were prepared post-printing, which the authors acknowledged as a potential limitation since one major advantage of 3D printed teeth is standardization, and the bias introduced by preparation was not considered. In contrast, Gok et al. prepared the natural tooth prior to micro-CT scanning, a method more suitable for obturation studies. Additionally, Peters et al. (2021) utilized 3D printed teeth derived from a previously prepared tooth to evaluate the quality of obturation among students (Peters et al., 2021).

Furthermore, it has been noted that removing support material from parts containing intricate features can be challenging (Fahad et al., 2013; Hulsmann, 2022; Orel et al., 2021; Reymus et al., 2019). No study has demonstrated the complete absence of support material within the internal root canal anatomy, with the majority failing to elucidate the protocol employed for its removal from inside the root canals, or whether such removal was undertaken at all (Cui et al., 2018; Gok et al., 2017; Karatekin et al., 2019; Kolling et al., 2021; Liang et al., 2018; Xu et al., 2021). This could result in partial or complete filling of the canals with support material, potentially impacting the variables of interest.

Reymus et al. (2019) conducted a retrograde preparation on the apical 5 mm of the natural tooth using hand files up to an ISO file 10 to ensure patency before a CBCT scan. Subsequently, all canals were prepared up to an ISO file 20. Following this, the access cavity was sealed with a cotton pellet and a radiopaque filling. Following printing with the Stereolithography technique, the 3D printed teeth underwent a 2-minute alcohol cleaning in an ultrasonic bath. Subsequently, they were centrifuged for 10 minutes at 3500 revolutions per minute to remove the uncured resin from the canals. The authors

emphasized the necessity of canal patency for effective uncured resin removal, hence the execution of retrograde preparation. However, the authors did not assess whether the canals were free of uncured resin following their protocol (Reymus et al., 2019).

In a study by Orel et al. (2021) comparing various preparation systems, it was noted that the apical 3 mm of the canals of 3D printed teeth, created using the Stereolithography technique, were obstructed by support material. Consequently, they fabricated another 3D printed tooth separated along the long axis of the tooth into two pieces, which were then secured together with a clamp (Orel et al., 2021). Given that the study aimed to assess the effects of different preparation systems on canal walls, the separation of teeth and their reliance on a clamp during the process may have introduced potential effects on the variables of interest that were not addressed by the authors.

Several studies examined the precision of 3D printed teeth by evaluating the external surface (Kulczyk et al., 2019; Liang et al., 2018; Reymus et al., 2019), while others focused on the internal root anatomy (Cui et al., 2018; Liang et al., 2018; Xu et al., 2021).

Reymus et al. (2019) solely examined the external surface of the 3D printed teeth. They compared the STL file of the natural tooth with STL files of the 3D printed teeth acquired through dental scanning. Although they suggested that the results might be comparable for the internal root anatomy, they did not conduct any assessment of this parameter(Reymus et al., 2019).

Liang et al. (2018) conducted a comparative study using CBCT to evaluate the precision of the external surface and internal root anatomy at 3, 6, and 9 mm from the apex in twenty natural premolars and their corresponding 3D printed teeth. The findings indicated no significant differences between the 3D printed teeth and the natural teeth (Liang et al., 2018). However, it's crucial to note that the natural teeth were categorized into four groups and prepared to different file sizes (25#06, 30#09, 35#04, and 40#40, respectively). Additionally, only three levels of the root canal were assessed. Therefore, these findings may not be extrapolated to teeth intended for studies or educational purposes involving intricate canal anatomy.

Cui et al. (2018) conducted a study in which they scanned six premolar teeth (three maxillary and three mandibular) and utilized these scans to create 3D printed teeth using the PolyJet technique. The support material was removed through melting, although the specific protocol was not detailed, and it was not specified whether the 3D printed teeth underwent access cavity preparation. The study concluded that the area and volume of the natural canals were not significantly different from those of the printed canals (Cui et al., 2018). However, for model validation, only one 3D printed tooth was compared with its respective natural tooth, resulting in six experimental pairs. Consequently, due to this methodology and the limited number of 3D printed teeth, it cannot be definitively concluded that the consistency of 3D printing internal root anatomy is maintained on a larger scale.

Xu et al. (2021) assessed the shaping ability of four single-file systems on 3D printed teeth created via the PolyJet technique. Micro-CT scans were conducted before and after preparation for each specimen (Xu et al., 2021). However, the authors failed to provide any details regarding the support material used. Another limitation is that, as the 3D

printed teeth were not compared with natural teeth, the reliability and consistency of the printing technique cannot be assured.

A primary concern highlighted in numerous studies is the discrepancy in radiopacity and hardness between the resin and dentin (Hulsmann, 2022; Karatekin et al., 2019; Kolling et al., 2021; Ordinola-Zapata et al., 2014; Reymus et al., 2019). In the study by Reymus et al. (2019), students perceived the preparation of 3D printed teeth to be easier than that of natural teeth, attributed to this hardness difference (Reymus et al., 2019). However, it's worth noting that the natural tooth underwent preparation up to an ISO file 20 before scanning to obtain the STL file, which could have also influenced the ease of preparation by the students.

In a study conducted by Cresswell-Boyes et al. (2022), their findings suggest that 3D printed typodont teeth, designed to mimic the mechanical properties of enamel and dentin, can exhibit mechanical comparability to extracted human teeth, despite variations in material compositions (Cresswell-Boyes et al., 2022). However, it's worth noting that they utilized a 50 µm layer printer and solely printed a typodont tooth without internal anatomy. While these results are promising, further research is warranted to explore the potential application of these materials in future endodontic studies involving 3D printed teeth.

Another consideration arises from the fact that the minimum layer thickness of 3D printers is 16 µm. However, it's worth pointing out that most apical foramen diameters typically fall within the range of 0.18 to 0.29 mm (Marroquin et al., 2004; Orel et al., 2021), suggesting that this limitation may not be significant. Nonetheless, reproducing smaller structures such as lateral canals and narrow isthmuses may pose challenges (Pouhaer et al., 2022; Reymus et al., 2019).

The process of producing 3D printed teeth comprises several steps, each of which presents potential sources of error (Kulczyk et al., 2019). A range of robust freeware and open-source software solutions exist for creating 3D printed teeth (Cui et al., 2018; Kulczyk et al., 2019; Reymus et al., 2020). However, the lack of standardization and established protocols fosters divergent measurement methods and study accuracies, thereby hindering the comparison of results across different studies (Cui et al., 2018).

Another consideration pertains to the resolution of scans required to achieve high-quality 3D printed teeth. Kulczyk et al. (2019) conducted a comparative analysis of the external surface of 3D printed teeth obtained through various 3D data acquisition methods, including CBCT, optical 3D scanning, and micro-CT. They concluded that while CBCT can provide sufficient data for 3D printed teeth, it does not match the precision of micro-CT. For instance, in their study, CBCT scans of a canine and a molar produced data with 65,340 and 98,624 triangles, respectively, at high resolution, whereas micro-CT scans yielded significantly higher numbers: 11,510,788 and 24,974,112 triangles for each tooth, respectively. As previously noted, higher resolution in the original data leads to better quality 3D models and subsequently, better quality 3D printed objects. However, 3D models created based on micro-CT data offer greater accuracy than current 3D printers can achieve.(Kulczyk et al., 2019). Additionally, very high-resolution STL files result in excessively large files that can pose challenges for 3D printing software to process.

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Therefore, in order to attain the required level of standardization and the development of protocols, addressing the issues surrounding 3D printed teeth as presented here should be prioritized.

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2. JUSTIFICATION AND AIMS

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2. JUSTIFICATION AND AIMS

2.1 JUSTIFICATION

Traditionally, the norm for ex vivo studies and pre-clinical courses has been to utilize extracted human teeth, which have long been regarded as the gold standard (Bitter et al., 2016; De-Deus et al., 2020; Decurcio et al., 2019; Dobros et al., 2022; Hulsmann, 2022; Reymus et al., 2019) and are considered the best for simulating the clinical setting, despite the lack of evidence to support this claim (Choi et al., 2021). Nonetheless, employing human teeth offers a valuable means of replicating clinical scenarios and grants students insight into internal anatomy, along with the tactile experience of dentine manipulation (Gancedo-Caravia et al., 2020). However, extracted human teeth has several drawbacks, including the difficulty of collection, ethical concerns, potential risks of cross-infection, storage limitations, and issues regarding standardization (Al-Sudani & Basudan, 2017; De-Deus, 2012; Holden & Dracopoulos, 2017; Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020).

Artificial teeth that reproduce the features of natural teeth may overcome these limitations (Al-Sudani & Basudan, 2017; Dobros et al., 2022; Reymus et al., 2021; Reymus et al., 2020). Over the past few years, various factory-manufactured models have emerged in the market; being realistic and standardized. However, their costs are high, they offer a restricted type of tooth options, the delivery times and a dependency on a manufacturer must be considered, also they exhibit substantial discrepancies in both manufacturing methods and materials across commercial brands (Hulsmann, 2022; Reymus et al., 2019; Reymus et al., 2020; Yekta-Michael et al., 2021).

Numerous studies employ 3D printed teeth; however, the establishment of standardized protocols remains crucial. Significant methodological variations persist among these studies, underscoring the need for cohesive standardization efforts (Decurcio et al., 2019; Hulsmann, 2022). The main objective behind employing 3D printed teeth is achieving standardization. For researchers, this creates the possibility of establishing equal and consistent protocols for ex vivo studies, enabling straightforward comparation of results. Keeping in mind that their internal anatomy should closely resemble that of the original natural teeth, their behaviour to preparation should mimic natural teeth and they should be effectively capable to evaluate and demonstrate differences between two preparation systems, also printing these teeth on a large scale, rapidly, and at minimal cost should be achievable.

Therefore, in order to achieve the requisite degree of standardization and the establishment of protocols, it is imperative to address the problems associated with 3D printed teeth.

Difficulty has been reported in removing support material from intricately detailed parts, potentially resulting in partial or complete filling of canals (Fahad et al., 2013; Hulsmann, 2022; Orel et al., 2021; Reymus et al., 2019). Therefore, there is the need to investigate

the influence of support material on the file action and its potential effects on the variables of interest. The development and validation of a protocol, for complete removing support material from 3D printed teeth. Subsequently, assessing the consistency and accuracy of the printing technique by comparing the internal anatomy of natural teeth with 3D printed teeth using micro-CT is essential.

2.2 AIMS

2.2.1 First primary aim

Evaluate the impact of support material of TrueTooth® (DELabs) replica, on the preparation technique and its potential effects on the volume increase evaluation using micro-CT scans.

2.2.1.1 Secondary aims

Evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, on TrueTooth® (DELabs) compared to natural teeth.

2.2.2 Second primary aim

Developing and validating a protocol for complete removing support material from 3D printed teeth and assessing its efficacy by comparing the internal anatomy of a natural tooth with 3D printed teeth using micro-CT scans.

2.2.2.1 Secondary aims

To assess the behaviour of natural tooth compared to 3D printed teeth, regarding preparation with ProTaper Gold® (Dentsply-Sirona) up to the F2 file. Comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

Evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system on natural tooth compared to 3D printed teeth.

To assess the behaviour of 3D printed teeth to preparation with ProTaper Gold® (Dentsply-Sirona) up to F2 file and Endogal® (Endogal Galician Endodontics Company) up to D file. Comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

Evaluate the time and number of pecking movements required for root canal preparation on 3D printed teeth using the ProTaper Gold® (Dentsply-Sirona) system compared to Endogal® (Endogal Galician Endodontics Company) system.

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3. HYPOTHESIS

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3. HYPOTHESIS

3.1 HYPOTHESIS OF FIRST PRIMARY AIM

The null hypothesis is that the TrueTooth® (DELabs) support material does not influence the evaluation of volume increase using micro-CT scans.

3.1.1 Hypothesis of secondary aim

The null hypothesis is that the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, are equal between TrueTooth® (DELabs) and natural teeth.

3.2 HYPOTHESIS OF SECOND PRIMARY AIM

The null hypothesis is that there is no difference between the internal anatomy of natural tooth and 3D printed teeth, demonstrating the efficacy of the support material removal protocol.

3.2.1 Hypothesis of secondary aims

The null hypothesis is that no difference would be observed between the behaviour of natural tooth compared to 3D printed teeth, when prepared with ProTaper Gold® (Dentsply-Sirona) up to the F2 file, when comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

The null hypothesis is that the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, are equal between natural tooth and 3D printed teeth.

The null hypothesis is that no difference would be observed between the behaviour of 3D printed teeth prepared with ProTaper Gold® (Dentsply-Sirona) up to F2 file compared to preparation with Endogal® (Endogal, Galician Endodontics Company) up to D file, when comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans.

The null hypothesis is that the time and number of pecking movements required for root canal preparation on 3D printed teeth are equal between the ProTaper Gold® (Dentsply-Sirona) system and the Endogal® (Endogal, Galician Endodontics Company) system.

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4. MATERIAL AND METHODS

Sections of this chapter has already been published as:

Reis, T., et al., Root Canal Preparation of a Commercial Artificial Tooth versus Natural Tooth - A MicroCT Study. Applied Sciences, 2023. 13(16): p. 9400.
https://doi.org/10.3390/app13169400

Reis, T., et al., Three-Dimensional Printed Teeth in Endodontics: A New Protocol for Microcomputed Tomography Studies. Materials. 2024; 17(8):1899.
https://doi.org/10.3390/ma17081899

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4. MATERIALS AND METHODS

4.1 MATERIAL AND METHODS FOR FIRST PRIMARY AIM

The study protocol was approved by the ethics committee of Instituto Asturiano de Odontologia of Oviedo University (IAO-23-0104) (Appendix 1)

Based on data from a previous study (Gagliardi et al., 2015) in which shaping ability was assessed a sample size calculation was performed using G*Power 3.1.9.7 software for windows (Heinrich Heine, Universität Düsseldorf, Germany) with an α type error of 0.05 and a β power of 0.95 and for an effect size of 1.6 (volume increase) input into the *t* test family, resulting in a required sample size of 20 samples (10 per group) to observe significant differences between groups. Fourteen samples were used per group to compensate for possible sample loss during experimental procedures. The concept of effect size in statistics refers to the magnitude of the effect associated with the studied phenomenon. It signifies the extent to which an event is prevalent within a specific population. Consequently, a larger effect size indicates a more pronounced manifestation of the studied event within the population, as expected in the effects of preparation on root canal volume increase (Gomes et al., 2021).

4.1.1 Natural teeth selection

Out of an initial pool of 65 maxillary permanent molars, which were extracted for reasons unrelated to this study, specimens were collected and preserved in distilled water until required. Radiographs were captured from both mesiodistal and buccolingual directions to ensure that the inclusion criteria were met. The inclusion criteria were teeth with fully formed apices, absence of root fractures, no signs of external and internal resorption or decayed tissue in the region of interest, absence of previous endodontic treatment, absence of calcifications, presence of separated canals and apical foramens, and a curvature degree ranging between 20° and 50°. The curvature degree was assessed using Schneider's method (Schneider, 1971), involving the drawing of two lines: one parallel to the canal's long axis, and the other from the apical foramen to intersect with the first line at the point where the canal deviated from the tooth's long axis.

According to these criteria, and to economize resources allocation, the buccal canals of 7 maxillary molars were selected. All the preparations were conducted by a single operator, the doctoral student, with 22 years of clinical experience in the field of endodontics with prior clinical experience in ProTaper Gold® (Dentsply Sirona) system use. The teeth were securely positioned using the ProTrain system® (Simit Dental, Mantova, Italy).

The endodontic cavities were prepared using a round diamond bur #4 and an Endo-Z™ bur (Dentsply Sirona) powered by a high-speed handpiece under water cooling. The buccal canals were explored by advancing passively into the canal a K-file #10 (Dentsply Sirona) until the file's tip was just visible through the apical foramen. The working length was established by subtracting 1 mm from this measurement.

4.1.2 Artificial specimen selection

In the artificial teeth group, fourteen TrueTooth® (DELabs) replicas (#15-001) were used. TrueTooth® replica #15-001 is a maxillary molar with a distobuccal canal with a severe curvature and a large mesiobuccal canal, ribbon-shaped, with a moderate curvature (DELabs); these features are adequate for the purposes of the present study. The 14 mesiobuccal canals were utilized to assess the effect of the support material, while the distobuccal canals served for comparison with the 14 natural buccal canals. The preparation of artificial teeth was equal to natural teeth.

4.1.3 Initial micro-CT scanning

The specimens underwent scanning with a micro-CT device (Skyscan 1174; Bruker) using 50 KV and 800 mA energy. A 0.25 mm thick aluminum filter was employed, with rotational steps of 1.0 increments covering a total rotation of 180º. Image pixel size was 16.65 µm, and the exposure time was set at 12000 ms. The images were reconstructed utilizing NRecon v 1.7.46 software (Bruker micro-CT), introducing the algorithms designed for correcting of ring artifacts (5), smoothing (3), and beam hardening (20%). The CTAnv v 1.20.3.0 software (Bruker micro-CT) was employed to calculate the initial volume. The region of interest was defined from the furcation region to the root apex.

The degree of homogeneity among the groups concerning the initial volume, working length, and degree of curvature was statistically evaluated and confirmed ($p > 0.05$), signifying adequate paring between natural teeth buccal canals and artificial teeth distobuccal canals.

4.1.4 Root Canal Preparation

An electric motor X-Smart® Plus (Dentsply Sirona) was used to operate the files with in-and-out pecking motion with an amplitude of 2–3 mm. A continuous clockwise rotation motion was used, according to the manufacturer's recommendations.

The number of pecking movements required for each file to reach the working length and the duration of file rotation within the canal until reaching the working length were recorded, using a start-stopwatch. If the working length wasn't reached within 3 pecking movements, the file was withdrawn from the canal to clean debris from the flutes. During this period, the start-stopwatch was paused.

A glyde path was established using a ProGlyder® file (Dentsply Sirona) until reaching the working length. Subsequently, all files from the ProTaper Gold® (Dentsply Sirona) system were employed up to the working length, following the sequence SX, S1, S2, F1, and F2. Patency was confirmed after each file's use with a K-file #10 (Dentsply Sirona). In all groups, the files were used to prepare only two canals before being replaced.

Between each file, root canal irrigation was carried out using 5.25% sodium hypochlorite (Cerkamed), as indicated by the TrueTooth® (DELabs) replicas developer (Buchanan, 2014), and an IrriFlex® 30G needle (Produits dentaires SA), inserted as apically as feasible. Each canal was irrigated with a total of 25 Ml. Subsequently, the canals were dried with absorbent paper points (Dentsply Sirona), followed by a new

micro-CT scan conducted under the same scanning and reconstruction parameters as initially established.

4.1.5 Micro-CT analysis

Images were reconstructed using the Nrecon v 1.7.46 software (Bruker micro-CT) and superimposed with geometric alignment using the DataViewer v 1.5.6.2 software (Bruker micro-CT). The CTAnv v 1.20.3.0 software (Bruker micro-CT) was employed to calculate the quantitative parameters. The region of interest was defined from the furcation region to the root apex.

The initial volume and final volume were acquired, with initial volume representing the preoperative volume of the root canals and the final volume representing the volume of the root canals after the preparation. Using these values, the root canal volume increase was calculated using the formula: final volume minus initial volume.

4.1.6 Total preparation time

The individual time required for each file to reach the working length were recorded and summed to determine the total preparation time needed for each canal.

4.1.7 Total number of pecking movements

The total number of pecking movements required for the preparation of each canal was calculated by summing the number of pecking movements needed for each file to reach the working length.

4.2 MATERIAL AND METHODS FOR SECOND PRIMARY AIM

The study protocol was approved by the ethics committee of Fernando Pessoa University (FCS/PI 429/23). (Appendix 2)

Based on data from a previous study (Gagliardi et al., 2015) in which shaping ability was assessed a sample size calculation was performed using G*Power 3.1.9.7 software for windows (Heinrich Heine, Universität Düsseldorf, Germany) with an α type error of 0.05 and a β power of 0.95 and for an effect size of of 1.6 (volume increase) and of 1.79 (percentage of unprepared area) input into the *t* test family. This calculation yielded a necessary sample size of 20 samples (10 per group) and 16 samples (8 per group) to detect significant differences between groups, respectively. Given that the maximum value obtained was ten samples per group, this number was utilized.

4.2.1 Natural tooth selection

Out of an initial pool of 45 maxillary permanent molars, which were extracted for reasons unrelated to this study, specimens were collected and preserved in distilled water until required. Radiographs were captured from both mesiodistal and buccolingual directions to ensure that the inclusion criteria were met. The inclusion criteria were teeth with fully formed apices, absence of root fractures, no signs of external and internal resorption or decayed tissue in the region of interest, absence of previous endodontic treatment, absence of calcifications, presence of separated canals and apical foramens,

and a curvature degree ranging between 20° and 40°. The curvature degree was assessed using Schneider's method (Schneider, 1971), involving the drawing of two lines: one parallel to the canal's long axis, and the other from the apical foramen to intersect with the first line at the point where the canal deviated from the tooth's long axis.

Out of the initial sample, 14 teeth were selected. The endodontic cavities were prepared using a round diamond bur #4 and an Endo-Z™ bur (Dentsply Sirona) driven by a high-speed handpiece under water cooling.
Exploration of the canals was conducted using a K-file #10 (Dentsply Sirona) until the file's tip was just visible through the apical foramen, ensuring canal patency. The teeth crowns were then sectioned 4mm from the cementoenamel junction to establish a platform for facilitating future references.

The specimens underwent scanning with a micro-CT device (Skyscan 1174; Bruker) using 50 KV and 800 mA energy. A 0.25 mm thick aluminum filter was employed, with rotational steps of 1.0 increments covering a total rotation of 180º. Image pixel size was 16.65 µm, and the exposure time was set at 12000 ms. The images were reconstructed utilizing NRecon v 1.7.46 software (Bruker micro-CT), introducing the algorithms designed for correcting of ring artifacts (3), smoothing (3), and beam hardening (40%).

CTAn v 1.20.3.0 software (Bruker micro-CT) was utilized to generate one STL file of each tooth and another for the respective canal anatomy. These STL files were then exported to a free software platform (MeshLab v. 2021.10) for qualitative evaluation of canal configuration and selection of the natural tooth to replicate.

The selected tooth was a second left maxillary molar featuring three fully separated roots, each presenting a single independent canal without any lateral canals. The mesiobuccal root exhibited an oval canal, while distobuccal root presented a small diameter round canal and palatal root presented a large diameter round canal (Figure 2).

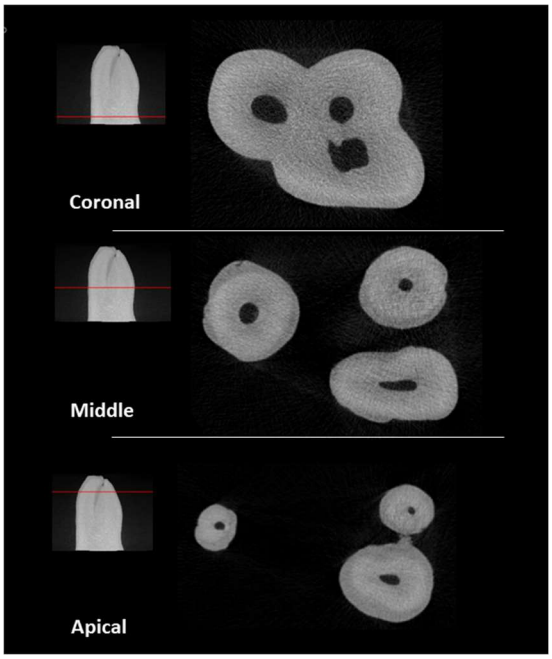

Figure 2. Cross-sectional views of micro-CT scan of the natural tooth (https://www.mdpi.com/about/openaccess)

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4.2.2 3D printing process and support material removal protocol

The STL file of the tooth were simplified and prepared for 3D printing using the "Simplification: Quadric Edge Collapse Decimation" filter (Moraes et al., 2019). This step was necessary as high-resolution STL files tend to be excessively large, posing challenges for 3D printing software to process (Reis et al., 2022).

Original STL file presented 3 298 498 vertices and 6 598 466 faces with a file size of 314 Mbytes. After simplification, the STL file presented 798 881 vertices and 1 599 232 faces with a file size of 76.2 Mbytes. The STL file was printed using PolyJet™ technology with a Stratasys Object30 Prime™ printer (Stratasys Ltd), in High-Quality mode featuring a layer thickness of 16 µm. High Temperature RGD525™ (Stratasys Ltd) was the printing material chosen, along with SUP706B™ (Stratasys Ltd) as the support material. For printing, the tooth was displayed horizontally with its mesial surface in contact with the printer bed, and its long axis aligned parallel to the X axis of printer head and bed (Figure 3).

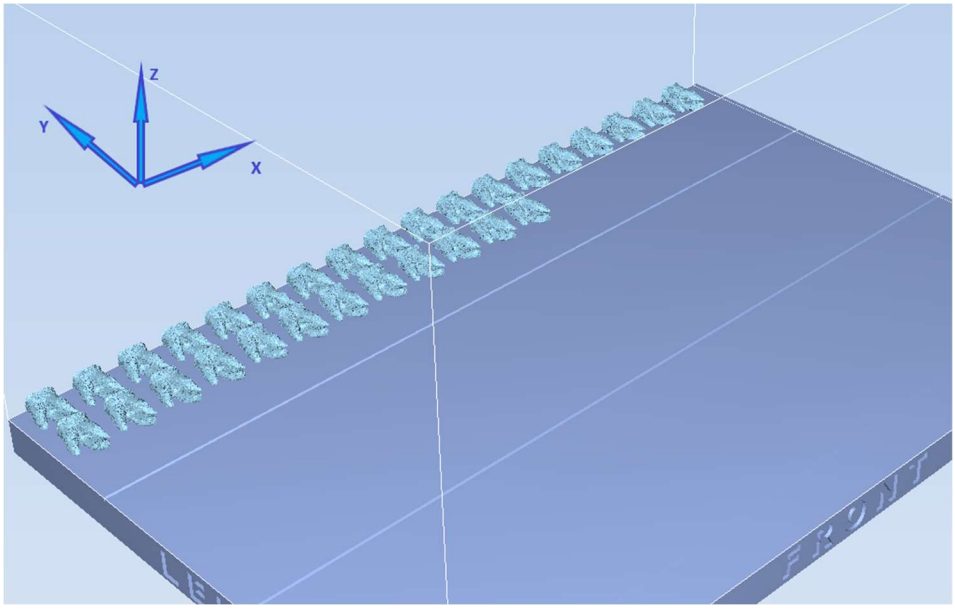

Figure 3. Teeth orientation on the printer bed (https://www.mdpi.com/about/openaccess)

Following printing, the support material surrounding the teeth and inside the access cavity was manually removed. The support material removal protocol, involved the use of a K-file #15 (Dentsply Sirona), a 30G polypropylene body needle Irriflex® (Produits dentaires SA), an Endoactivator® (Dentsply Sirona) with a small Endoactivator® Tip (15/.02) (Dentsply Sirona). Irrigants used in the process included a 5% solution of sodium hydroxide, 5 % Derquim® LM 01 alkalin detergent (ITW Reagents, S.R.L.), distilled water and 70% alcohol.

The support material removal protocol, summarized in a flowchart (Figure 4), consisted on the following steps: (1) cleaning the access cavity with 5 ml of 5% sodium hydroxide, and for each canal, (2) advancing passively into the canal the K-file #15 until the tip of the file was just visible through the apical foramen to ensure canal patency, followed by irrigation with 5 ml of 5% sodium hydroxide with high pressure. This step was repeated 5 times to achieve irrigant extrusion by the end of this step. This was

followed by (3) irrigation with 35 ml of 5% sodium hydroxide. During this step, if there was loss of irrigant extrusion, indicating a loss of patency, a K-file #15 was used as previously mentioned, (4) the irrigant was subjected to sonic activation with Endoactivator® (Dentsply Sirona) at high frequency for 30 seconds with up-and-down movements, with an amplitude of 4 mm. Following this, (5) the canal was irrigated with 5 ml of 5% sodium hydroxide, (6) followed by another round of irrigant sonic activation as previously described. This process continued with (7) irrigation using 5 ml of 5 % Derquim® LM 01 alkalin detergent (ITW Reagents, S.R.L.), (8) subsequent irrigant sonic activation as described before, (9) irrigation with 5 ml of distilled water, (10) additional irrigant sonic activation as described before, (11) irrigation with 5 ml of distilled water, and finally (12) irrigation with 2 ml of 70% alcohol.

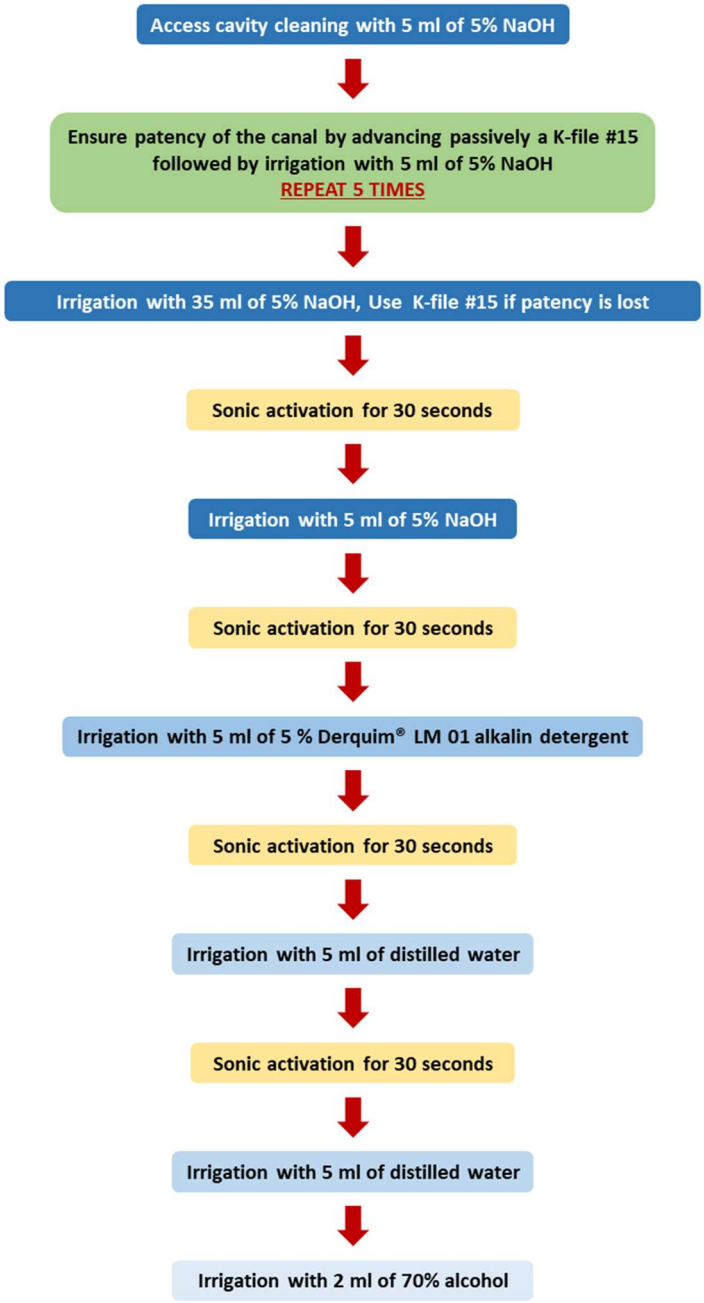

Figure 4. Flowchart of support material removal protocol (https://www.mdpi.com/about/openaccess)

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Thus, a total of 20 3D printed teeth sample (n=20) were obtained and randomly assigned (www.random.org) to each of the two experimental groups (n=10), ProTaper Gold® (Dentsply Sirona) and Endogal® (Endogal, Galician Endodontics Company) (Figure 5).

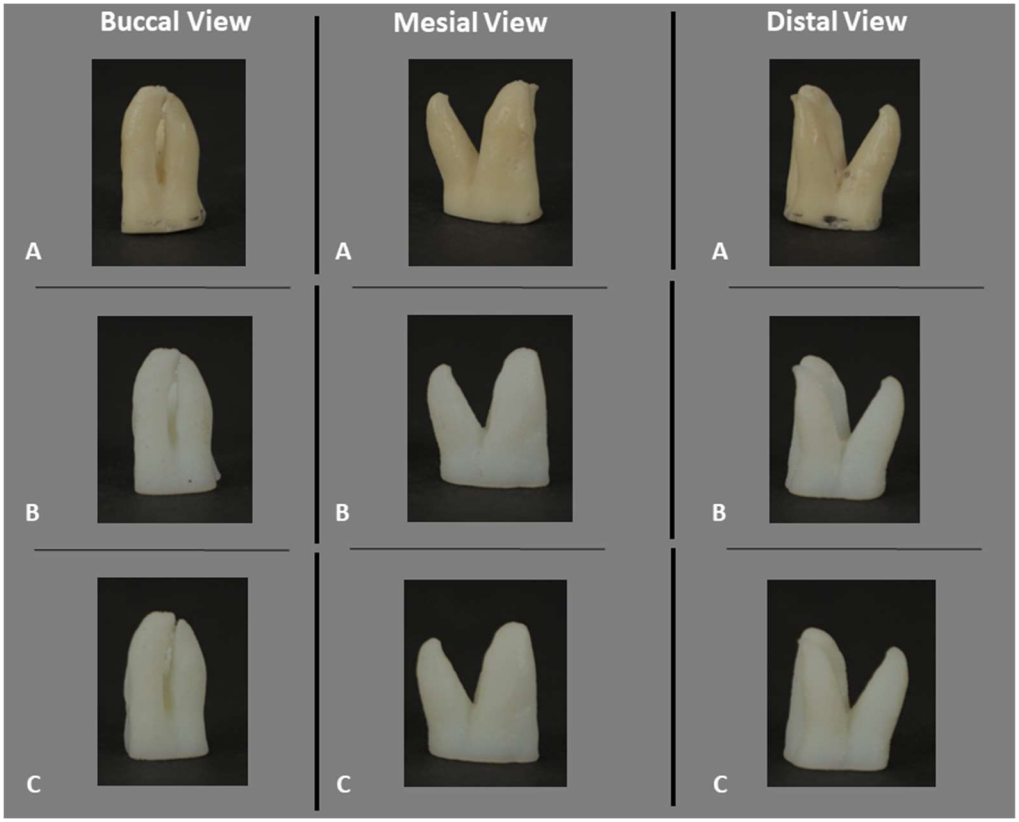

Figure 5. Natural tooth (A) and 3D printed teeth (B and C) from different views (https://www.mdpi.com/about/openaccess)

4.2.3 3D printed teeth initial micro-CT scanning

The specimens underwent scanning with a micro-CT device (Skyscan 1174; Bruker) using 50 KV and 800 Ma energy, with rotational steps of 1.0 increments covering a total rotation of 180º. Image pixel size was 16.65 µm, and the exposure time was set at 12000 ms. The images were reconstructed utilizing NRecon v 1.7.46 software (Bruker micro-CT), introducing the algorithms designed for correcting of ring artifacts (5), smoothing (3), and beam hardening (50%).

4.2.4 Root canal preparation

All the preparations were performed by a single operator, the doctoral student, with 22 years of clinical experience in the field of endodontics and with previously clinically experienced in ProTaper Gold® (Dentsply Sirona) and Endogal® (Endogal, Galician Endodontics Company) systems. The teeth were secured in position using the ProTrain system® (Simit Dental Srl). The working length was determined by subtracting 1 mm from the value obtained during the support material removal protocol.

An X-Smart® Plus electric motor (Dentsply Sirona) was used to operate the files employing in-and-out pecking motion with an amplitude of 2-3 mm, in a continuous clockwise rotation according to the manufacturer's recommendations.

The number of pecking movements required for each file to reach the working length and the duration of file rotation within the canal until reaching the working length were recorded, using a start-stopwatch. If the working length wasn't reached within 3 pecking movements, the file was withdrawn from the canal to clean debris from the flutes. During this period, the start-stopwatch was paused.

4.2.4.1 Preparation of natural tooth and group I with ProTaper Gold®

A glyde path was established using a ProGlyder® File (Dentsply Sirona) until reaching the working length. Subsequently, all files from the ProTaper Gold® (Dentsply Sirona) system were employed up to the working length except the SX file that was used only for coronal interferences removal, following the sequence SX, S1, S2, F1, and F2. Patency was confirmed after each file's use with a K-file #10 (Dentsply Sirona). The files were used to prepare only one tooth before being replaced.

Root canal irrigation was conducted between each file, using 5ml 5,25% NaOCl (Cerkamed) for NT and 5 ml distilled water for 3DPT, with an IrriFlex® 30G needle (Produits dentaires SA), inserted as apically as feasible, activated with Endoactivator® at high frequency with the small tip for 30 seconds. After preparation the canals were irrigated twice with 5 ml of irrigant and activated with Endoactivator® (Dentsply Sirona). Consequently, the total volume of irrigation for each canal was 40 ml. Finally the canals were dried with paper points (Dentsply Sirona).

4.2.4.2 Preparation of group II with Endogal®

A glyde path was established by using the A File (Endogal, Galician Endodontics Company) until working length was reached. All files from the Endogal® (Endogal, Galician Endodontics Company) system, were employed up to the WL except the X file that was used only for coronal interferences removal, following the sequence X , B, C and D. Patency was confirmed after each file's use with a K-file #10 (Dentsply Sirona). The files were used to prepare one tooth before being replaced.

Root canal irrigation was conducted between each file with 5ml distilled water using a Irriflex® 30G needle (Produits dentaires SA), inserted as apically as feasible, and activated with Endoactivator® at high frequency with the small tip for 30 seconds. After preparation the canals were irrigated three times with 5 ml of irrigant and activated with Endoactivator® (Dentsply Sirona).. Consequently, the total volume of irrigation for each canal was 40 ml. Finally the end the canals were dried with paper points (Dentsply Sirona).

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4.2.5 Micro-CT analysis

A new micro-CT was performed on natural tooth and both 3D printed teeth groups according to the same scanning and reconstruction parameters as those established initially respectively.

Images before and after preparation were superimposed with the 3D registration application of the DataViewer v 1.5.6.2 software (Bruker micro-CT). Subsequently, the data obtained were processed using CTAnv v 1.20.3.0 software (Bruker micro-CT). The region of interest was set from the furcation region to root apex.

The volume of dentine removed, and centroids were quantified by subtracting the values before preparation to the values after preparation (Hulsmann, 2022). Regarding the sample orientation during evaluation, a positive value for alteration of centroid X indicated a shift in buccal direction, centroid Y in mesial direction and centroid Z in apical direction (Perez Morales et al., 2021). Transportation was examined by calculating the vectorial translocation of all sections X, Y and Z coordinate values using the following formula, where "a" represents after and "b" before preparation: $\sqrt{(Xa\text{-}Xb)^2+(Ya\text{-}Yb)^2+(Za\text{-}Zb)^2}$ (Serefoglu & Piskin, 2017; Ventura et al., 2024).

The percentage of unprepared area was determined by comparing the number of static voxels with the total number of voxels present on root canal surface (Perez Morales et al., 2021). Tooth volume expansion evaluated for the 3D printed teeth, was expressed as the ratio of 3D printed teeth volume to the natural tooth volume (Lee et al., 2015).

4.2.6 Total preparation time

The individual time required for each file to reach the working length were recorded and summed to determine the total preparation time needed for each canal.

4.2.7 Total number of pecking movements

The total number of pecking movements required for the preparation of each canal was calculated by summing the number of pecking movements needed for each file to reach the working length.

4.3 STATISTICAL ANALYSIS

The data collected were processed using the IBM SPSS Statistics vs. 26.0 software. The Shapiro-Wilk test was applied to verify data normality. Accordingly, to normal and non-normal distribution, one-sample t-test or one-sample Wilcoxon signed rank test and Independent-Sample t-test or Mann-Whitney U test were applied. The significance level was 5% for all statistical tests ($p < 0.05$).

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5. RESULTS

Sections of this chapter has already been published as:

Reis, T., et al., Root Canal Preparation of a Commercial Artificial Tooth versus Natural Tooth - A MicroCT Study. Applied Sciences, 2023. 13(16): p. 9400.
https://doi.org/10.3390/app13169400

Reis, T., et al., Three-Dimensional Printed Teeth in Endodontics: A New Protocol for Microcomputed Tomography Studies. Materials. 2024; 17(8):1899.
https://doi.org/10.3390/ma17081899

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5. RESULTS

5.1 RESULTS OF FIRST PRIMARY AIM

Table 1 presents the results associated to the first primary aim, displaying the mean, standard deviation, median, minimum, and maximum values of the initial root volume, final root volume, and root canal volume increase, assessed by Micro-Ct scans, of the artificial teeth mesiobuccal canals, aiming to access the effect of the support material. It should be noted that the mean values for volume increase are negative, respectively -1.32 mm^3 and -17.66%.

Figure 6 illustrates the 3D analysis of the mesiobuccal root canal before preparation of an artificial tooth, while Figure 7 displays the same root after preparation. For comparative purposes, Figures 8 and 9 displays a mesiobuccal root canal of a natural tooth before and after preparation, respectively.

Table 1. Micro-CT analysis before and after preparation with ProTaper Gold® (Dentsply-Sirona) of artificial teeth Mesiobuccal Canal (https://www.mdpi.com/about/openaccess)

		Artificial Teeth Mesiobuccal Canal
Initial canal volume (mm^3)	Mean ± SD	7.45 ± 0.42
	Median	7.45
	Min-Max	7.00-8.05
Final canal volume (mm^3)	Mean ± SD	6.14 ± 0.6
	Median	5.93
	Min-Max	5.30-7.86
Canal volume increase (mm^3)	Mean ± SD	-1.32 ± 0.82
	Median	-1.58
	Min-Max	-2.14-0.86
Canal volume increase (%)	Mean ± SD	-17.66 ± 8.72
	Median	-20.48
	Min-Max	-28.89-5.41

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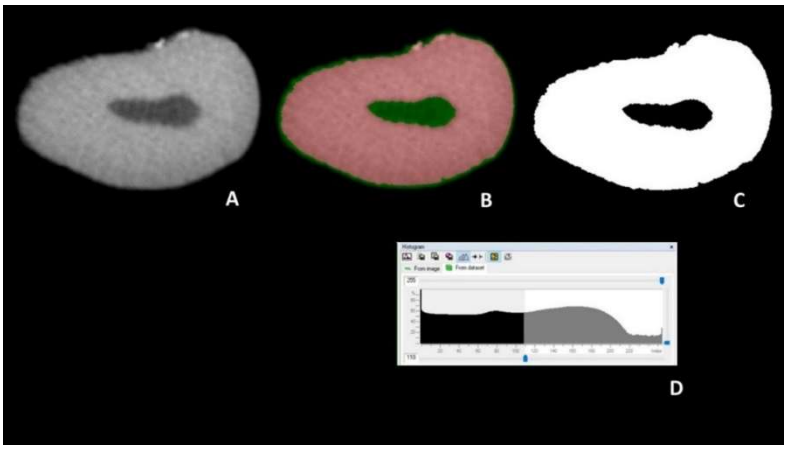

Figure 6. Artificial tooth mesiobuccal root before preparation: (A) raw image from micro-CT dataset; (B) image with threshold applied; (C) binarized black and white image; (D) histogram with selected threshold values (https://www.mdpi.com/about/openaccess)

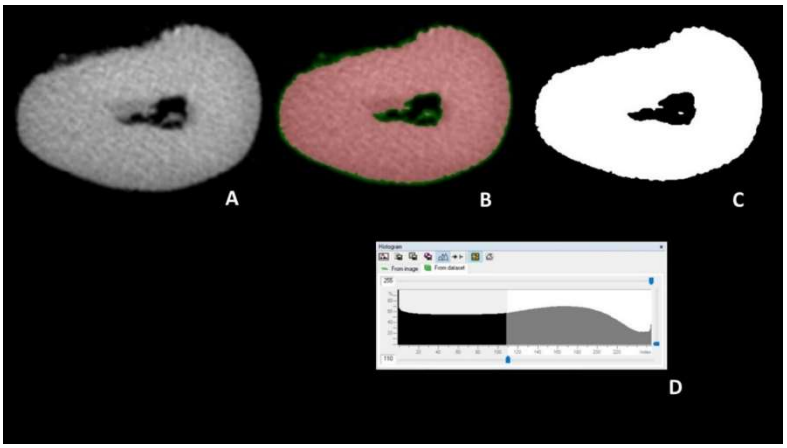

Figure 7. Artificial tooth mesiobuccal root after preparation: (A) raw image from micro-CT dataset; (B) image with threshold applied; (C) binarized black and white image; (D) histogram with selected threshold values (https://www.mdpi.com/about/openaccess)

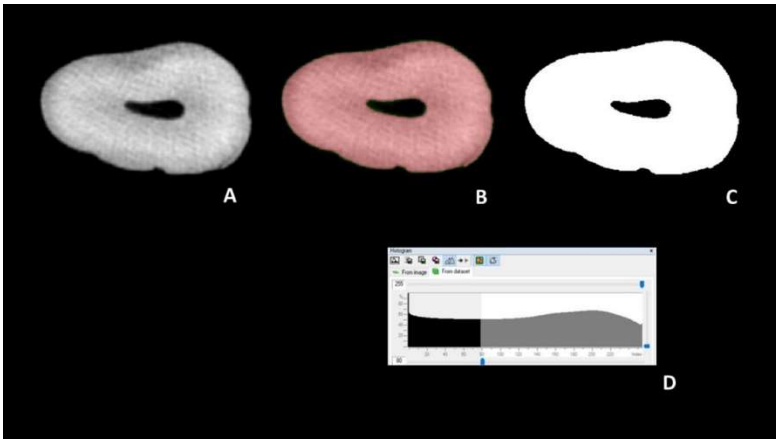

Figure 8. Natural tooth mesiobuccal root before preparation: (A) raw image from micro-CT dataset; (B) image with threshold applied; (C) binarized black and white image; (D) histogram with selected threshold values (https://www.mdpi.com/about/openaccess)

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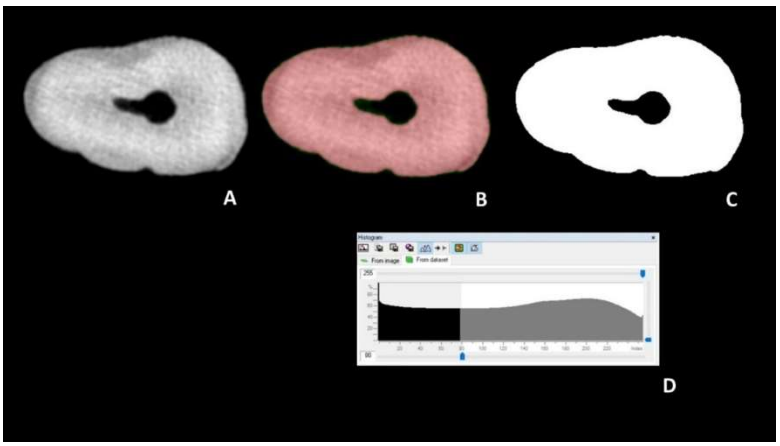

Figure 9. Natural tooth mesiobuccal root after preparation: (A) raw image from micro-CT dataset; (B) image with threshold applied; (C) binarized black and white image; (D) histogram with selected threshold values (https://www.mdpi.com/about/openaccess)

Table 2 presents the results, displaying the mean, standard deviation, median, minimum, and maximum values, of the initial root volume, final root volume, and root canal volume increase, assessed by Micro-Ct scans for both natural teeth buccal canals and artificial teeth distobuccal canals, to confirm the impact of support material. It should be noted the existence of negative values in the artificial teeth group.

Table 2. Micro-CT analysis before and after preparation with ProTaper Gold® (Dentsply-Sirona) of natural teeth versus artificial teeth (https://www.mdpi.com/about/openaccess)

		Natural Teeth	Artificial Teeth
Initial canal volume (mm^3)	Mean ± SD	2.68 ± 1.89	2.70 ± 0.11
	Median	2.29	2.70
	Min-Max	0.52–7.44	2.56–2.82
Final canal volume (mm^3)	Mean ± SD	4.07 ± 2.18	3.40 ± 0.68
	Median	3.48	3.64
	Min-Max	1.42–9.91	2.08–4.15
Canal volume increase (mm^3)	Mean ± SD	1.39 ± 0.67	0.71 ± 0.70
	Median	1.35	0.98
	Min-Max	0.34–2.77	-0.74–1.51
Canal volume increase (%)	Mean ± SD	88.09 ± 131.27	32.94 ± 26.51
	Median	47.52	42.47
	Min-Max	18.61–531.43	-18.56–62.49

Table 3 and Table 4 presents the results, displaying the mean, standard deviation, median, minimum, and maximum values, respectively, of the total and number of pecking movements for both natural teeth buccal canals and artificial teeth distobuccal canals, along with the time and number of pecking movements required for each file of the ProTaper Gold® (Dentsply Sirona) system.

The time required for preparation of natural teeth was slightly higher compared to artificial teeth, with 64.3 seconds versus 63.5 seconds.
However, no statistically significant differences were observed between natural teeth and artificial teeth in terms of the total time of preparation ($p > 0.05$), or for each individual file ($p > 0.05$).

The number of pecking movements required for preparation of natural teeth was higher compared to artificial teeth, with 33.5 movements versus 25.86 movements.
However, no statistically significant differences were observed between natural teeth and artificial teeth in terms of the total number of pecking movements ($p > 0.05$), or for each individual file ($p > 0.05$).

Table 3. Time of preparation in seconds for natural teeth and artificial teeth with ProTaper Gold® (Dentsply-Sirona)

Time of preparation		Natural Teeth	Artificial Teeth
ProGlyder®	Mean ± SD	12.21 ± 2.08	12.21 ± 1.19
	Median	12.00	12.50
	Min-Max	10-16	10-14
ProTaper Gold® Sx	Mean ± SD	8.64 ± 2.06	8.50 ± 1.02
	Median	8.00	9.00
	Min-Max	5-13	7-10
ProTaper Gold® S1	Mean ± SD	12.00 ± 2.08	11.79 ± 2.72
	Median	12.00	11.50
	Min-Max	9-16	9-18
ProTaper Gold® S2	Mean ± SD	10.64 ± 1.99	10.57 ± 1.16
	Median	11.00	10.50
	Min-Max	8-14	9-12
ProTaper Gold® F1	Mean ± SD	9.36 ± 2.44	9.29 ± 1.44
	Median	8.00	9.50
	Min-Max	6-14	6-11
ProTaper Gold® F2	Mean ± SD	11.43 ± 2.50	11.21 ± 1.48
	Median	11.00	12.00
	Min-Max	8-16	7-13
Total	Mean ± SD	64.3 ± 10.67	63.50 ± 4.85
	Median	62.00	63.00
	Min-Max	52-87	51-71

Table 4. Number of pecking movements for natural teeth and artificial teeth with ProTaper Gold® (Dentsply-Sirona)

Number of pecking movements		Natural Teeth	Artificial Teeth
ProGlyder®	Mean ± SD	6.43 ± 0.76	4.57 ± 2.71
	Median	7.00	4.00
	Min-Max	5-7	2-8
ProTaper Gold® Sx	Mean ± SD	4.93 ± 0.83	3.86 ± 2.07
	Median	5.00	3.50
	Min-Max	4-6	2-8
ProTaper Gold® S1	Mean ± SD	6.64 ± 1.28	5.07 ± 2.34
	Median	7.00	5.00
	Min-Max	4-9	2-8
ProTaper Gold® S2	Mean ± SD	5.50 ± 1.16	4.43 ± 1.91
	Median	6.00	4.50
	Min-Max	4-7	2-7
ProTaper Gold® F1	Mean ± SD	4.21 ± 0.80	3.50 ± 1.65
	Median	4.00	3.00
	Min-Max	3-5	2-6
ProTaper Gold® F2	Mean ± SD	5.79 ± 0.58	4.43 ± 2.47
	Median	6.00	4.00
	Min-Max	5-7	2-8
Total	Mean ± SD	33.50 ±4.00	25.86 ± 12.75
	Median	34.50	24.50
	Min-Max	26-38	12-44

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5.2 RESULTS OF SECOND PRIMARY AIM

Table 5 presents the results associated to the second primary aim, displaying the mean, standard deviation, median, minimum, and maximum values of canal volume, centroid X, Y, Z before preparation for both natural tooth and 3D printed teeth, and percentage of tooth volume expansion for the 3D printed teeth, assessed by Micro-CT scans. These results aim to evaluate the effectiveness of the support material removal protocol. There were no statistically significant differences between natural tooth and 3D printed teeth for all variables ($p > 0.05$) (Figure 10 and 11).

Table 5. Micro-CT analysis before preparation of natural tooth and 3D printed teeth (https://www.mdpi.com/about/openaccess)

	Natural Tooth	3D Printed Teeth	
Canal Volume (mm^3)	12.08	Mean ± SD	11.99 ± 0.55
		Median	12.07
		Min-Max	11.03-13.13
Centroid X (mm)	10.92	Mean ± SD	10.92 ± 0.09
		Median	10.93
		Min-Max	10.70-11.04
Centroid Y (mm)	10.37	Mean ± SD	10.35 ± 0.07
		Median	10.35
		Min-Max	10.24-10.46
Centroid Z (mm)	8.03	Mean ± SD	8.02 ± 0.09
		Median	8.04
		Min-Max	7.70-8.13
Tooth Volume Expansion (%)		Mean ± SD	0.73 ± 0.77
		Median	0.68
		Min-Max	0.01-2.38

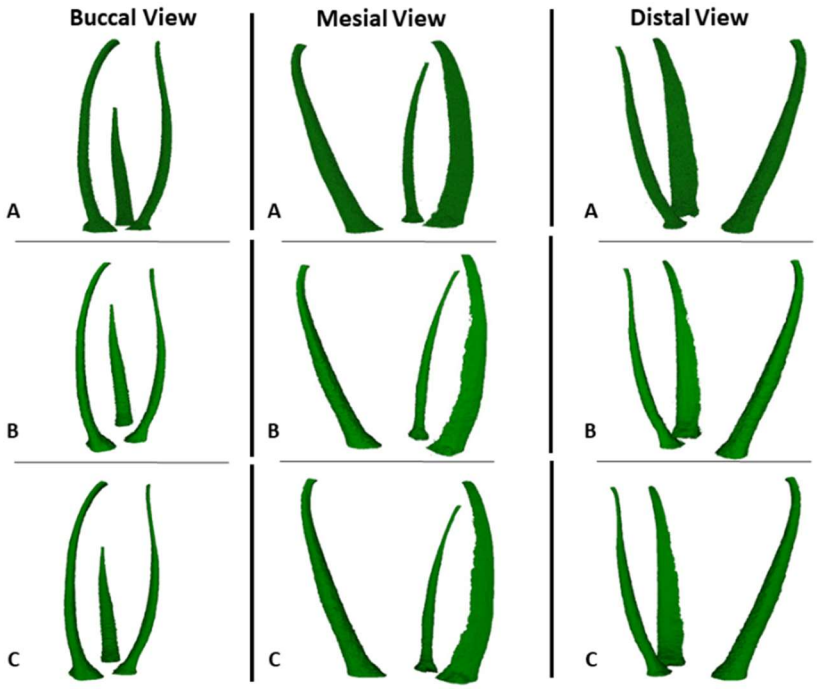

Figure 10. Representative 3D reconstruction of micro-CT scans before preparation from different views. (A) natural tooth, (B) 3D printed teeth of ProTaper Gold® (Dentsply-Sirona) group and (C) 3D printed teeth of Endogal® (Endogal, Galician Endodontics Company) group (https://www.mdpi.com/about/openaccess)

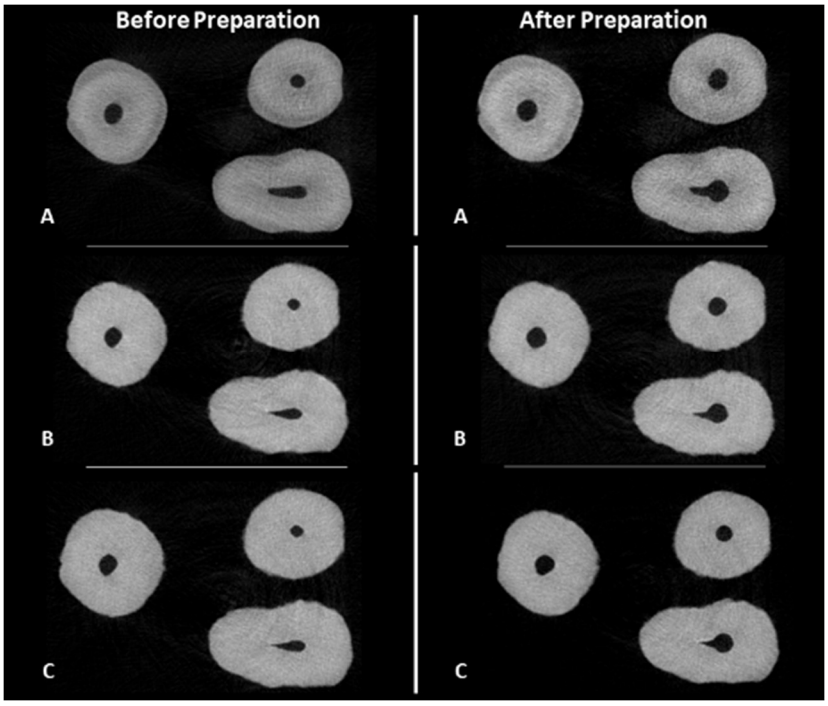

Figure 11. Cross-sectional views of micro-CT scans before and after preparation. (A) natural tooth, (B) 3D printed teeth of ProTaper Gold® (Dentsply-Sirona) group and (C) 3D printed teeth of Endogal® (Endogal, Galician Endodontics Company) group (https://www.mdpi.com/about/openaccess)

Table 6 and Table 7 presents the results, displaying the mean, standard deviation, median, minimum, and maximum values, respectively, of the volume increase, volume of dentin removed, centroids, transportation, unprepared areas assessed by micro-CT scans, and the time of preparation and number of pecking movements required for root canal preparation, for both natural tooth and 3D printed teeth, along with the time and number of pecking movements required for each file of the ProTaper Gold® (Dentsply Sirona) system.

There were no statistically significant differences between natural tooth and 3D printed teeth for all variables before preparation ($p > 0.05$). The 3D printed teeth showed a similar behaviour to natural tooth after preparation, since there were no statistically significant differences ($p > 0.05$), related to the measurements of canal volume, centroid X, Y, Z after preparation, and to volume of dentin removed, percentage of volume increase, centroid X, Y, Z alteration, transportation, and percentage of unprepared areas (Figure 11 and 12).

The time required for preparation of natural tooth was slightly higher compared to 3D printed teeth, with 179 seconds versus 177 seconds.
However, no statistically significant differences were observed between natural tooth and 3D printed teeth in terms of the total time of preparation ($p > 0.05$), or for each individual file ($p > 0.05$).

The number of pecking movements required for preparation of natural tooth was higher compared to 3D printed teeth, with 102 movements versus 93 movements.

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However, no statistically significant differences were observed between natural tooth and 3D printed teeth in terms of the total number of pecking movements ($p > 0.05$), or for each individual file ($p > 0.05$).

Table 6. Micro-CT analysis before and after preparation with ProTaper Gold® (Dentsply-Sirona) of natural tooth and 3D printed teeth (https://www.mdpi.com/about/openaccess)

		Natural Tooth	3D Printed Teeth	
Canal Volume (mm^3)	Initial	12.08	Mean ± SD Median Min-Max	12.12 ± 0.42 12.12 11.24-12.90
	After	17.75	Mean ± SD Median Min-Max	17.89 ± 0.69 17.66 17.16-18.94
	Volume of dentin removed	5.68	Mean ± SD Median Min-Max	5.77 ± 0.66 5.67 4.92-7.11
	% Volume Increase	46.99	Mean ± SD Median Min-Max	47.68 ± 6.09 45.50 40.1-60.1
Centroid X (mm)	Initial	10.92	Mean ± SD Median Min-Max	10.92 ± 0.07 10.87 10.85-11.04
	After	11.38	Mean ± SD Median Min-Max	11.33 ± 0.13 11.32 11.12-11.54
	Alteration	0.46	Mean ± SD Median Min-Max	0.41 ± 0.17 0.41 0.20-0.69
Centroid Y (mm)	Initial	10.37	Mean ± SD Median Min-Max	10.37 ± 0.07 10.40 10.27-10.44
	After	10.50	Mean ± SD Median Min-Max	10.53 ± 0.04 10.53 10.47-10.60
	Alteration	0.13	Mean ± SD Median Min-Max	0.16 ± 0.07 0.15 0.05-0.27
Centroid Z (mm)	Initial	8.03	Mean ± SD Median Min-Max	8.01 ± 0.11 8.04 7.70-8.09
	After	8.10	Mean ± SD Median Min-Max	8.01 ± 0.10 8.02 7.84-8.20
	Alteration	0.07	Mean ± SD Median Min-Max	0.01 ± .015 0.02 -0.25-0.27
Transportation (mm)		0.48	Mean ± SD Median Min-Max	0.48 ± 0.14 0.45 0.30-0.73
Unprepared area (%)		57.64	Mean ± SD Median Min-Max	54.97 ± 3.79 53.88 51.13-62.93

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Table 7. Time of preparation in seconds and number of pecking movements for natural tooth and 3D printed teeth with ProTaper Gold® (Dentsply-Sirona)

	Natural Tooth			3D Printed Teeth	
	Time of Preparation	Number of pecking movements		Time of Preparation	Number of pecking movements
ProGlyder®	27	17	Mean ± SD Median Min-Max	27.30 ± 3.86 27.00 21-33	14.70 ± 6.24 18.00 6-21
ProTaper Gold® Sx	27	18	Mean ± SD Median Min-Max	25.80 ± 3.80 25.50 21-33	15.00 ± 7.07 16.50 6-27
ProTaper Gold® S1	33	17	Mean ± SD Median Min-Max	32.70 ± 4.35 33.00 27-39	15.30 ± 4.35 18.00 9-18
ProTaper Gold® S2	31	19	Mean ± SD Median Min-Max	32.10 ± 2.47 31.50 30-36	17.10 ± 3.76 18.00 12-21
ProTaper Gold® F1	27	13	Mean ± SD Median Min-Max	27.90 ± 4.25 27.00 24-36	13.20 ± 5.69 13.50 6-21
ProTaper Gold® F2	34	18	Mean ± SD Median Min-Max	31.20 ± 5.14 30.00 21-39	17.70 ± 8.30 22.50 6-24
Total	179	102	Mean ± SD Median Min-Max	177.00 ± 8.72 175.50 165-195	93.00 ± 34.41 103.50 45-132

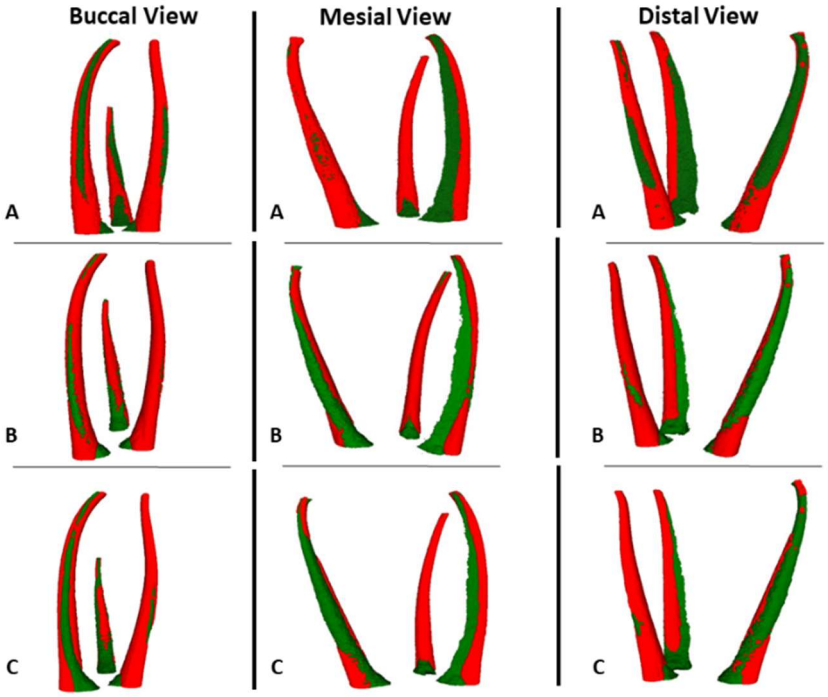

Figure 12. Representative 3D reconstruction of micro-CT scans before (green) and after (red) preparation from different views. (A) natural tooth, (B) 3D printed teeth of ProTaper Gold® (Dentsply-Sirona) group and (C) 3D printed teeth of Endogal® (Endogal, Galician Endodontics Company) group (https://www.mdpi.com/about/openaccess)

Table 8, Table 9, and Table 10 presents the results, displaying the mean, standard deviation, median, minimum, and maximum values, respectively, of the volume increase, volume of dentin removed, centroids, transportation, unprepared areas assessed by micro-CT scans, and the time of preparation and number of pecking movements required for root canal preparation, for both ProTaper Gold® (Dentsply Sirona) and Endogal® (Endogal, Galician Endodontics Company) systems, along with the time and number of pecking movements required for each file of each system.

There were no statistically significant differences observed for all variables before preparation ($p > 0.05$). Both systems exhibited similar behavior, as indicated by the absence of statistically significant differences ($p > 0.05$) in measurements of canal volume, centroid X, Y, Z after preparation, volume of dentin removed, percentage of volume increase, centroid X, Y, Z alteration, transportation, and percentage of unprepared areas (Figure 11 and 12).

However, the ProTaper Gold® (Dentsply Sirona) system demonstrated a higher percentage of volume increase and a lower percentage of untouched areas, at 47.68% and 54.97%, respectively, compared to the Endogal® (Endogal, Galician Endodontics Company) system, which showed percentages of 45.02% and 56.41%, respectively.

The time required for preparation with ProTaper Gold® (Dentsply Sirona) system was higher compared to Endogal® (Endogal, Galician Endodontics Company) system with 177 seconds versus 111.6 seconds.

Statistically significant differences were observed between the two systems regarding the total time of preparation ($p < 0.05$). Additionally, statistically significant differences were observed between the ProGlyder® (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company) ($p < 0.05$).

The number of pecking movements required for preparation with ProTaper Gold® (Dentsply Sirona) system was higher compared to Endogal® (Endogal, Galician Endodontics Company) system with 93 movements versus 47.40 movements.

Statistically significant differences were observed between the two systems regarding the number of pecking movements ($p < 0.05$). Additionally, statistically significant differences were observed between the ProGlyder® (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company) ($p < 0.05$).

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Table 8. Micro-CT analysis before and after preparation with ProTaper Gold® (Dentsply-Sirona) and Endogal® (Endogal, Galician Endodontics Company) of 3D printed teeth (https://www.mdpi.com/about/openaccess)

			ProTaper Gold®	Endogal®
Canal Volume (mm^3)	Initial	Mean ± SD	12.12 ± 0.42	11.86 ± 0.66
		Median	12.12	11.82
		Min-Max	11.24-12.90	11.03-13.13
	After	Mean ± SD	17.89 ± 0.69	17.02 ± 0.61
		Median	17.66	17.06
		Min-Max	17.16-18.94	15.68-17.93
	Volume of dentin removed	Mean ± SD	5.77 ± 0.66	5.24 ± 1.02
		Median	5.67	5.49
		Min-Max	4.92-7.11	3.46-6.48
	% Volume Increase	Mean ± SD	47.68 ± 6.09	45.02 ± 10.89
		Median	45.50	47.25
		Min-Max	40.1-60.1	28.29-58.77
Centroid X (mm)	Initial	Mean ± SD	10.92 ± 0.07	10.92 ± 0.11
		Median	10.87	10.96
		Min-Max	10.85-11.04	10.70-11.02
	After	Mean ± SD	11.33 ± 0.13	11.37 ± 0.06
		Median	11.32	11.35
		Min-Max	11.12-11.54	11.30-11.47
	Alteration	Mean ± SD	0.41 ± 0.17	0.45 ± 0.09
		Median	0.41	0.43
		Min-Max	0.20-0.69	0.34-0.63
Centroid Y (mm)	Initial	Mean ± SD	10.37 ± 0.07	10.33 ± 0.06
		Median	10.40	10.32
		Min-Max	10.27-10.44	10.24-10.46
	After	Mean ± SD	10.53 ± 0.04	10.44 ± 0.05
		Median	10.53	10.45
		Min-Max	10.47-10.60	10.33-10.50
	Alteration	Mean ± SD	0.16 ± 0.07	0.11 ± 0.05
		Median	0.15	0.13
		Min-Max	0.05-0.27	0.02-0.16
Centroid Z (mm)	Initial	Mean ± SD	8.01 ± 0.11	8.03 ± 0.07
		Median	8.04	8.01
		Min-Max	7.70-8.09	7.94-8.13
	After	Mean ± SD	8.01 ± 0.10	8.07 ± 0.09
		Median	8.02	8.11
		Min-Max	7.84-8.20	7.89-8.14
	Alteration	Mean ± SD	0.01 ± .015	0.05 ± 0.09
		Median	0.02	0.02
		Min-Max	-0.25-0.27	-0.09-0.19
Transportation (mm)		Mean ± SD	0.48 ± 0.14	0.49 ± 0.11
		Median	0.45	0.47
		Min-Max	0.30-0.73	0.38-0.69
Unprepared area (%)		Mean ± SD	54.97 ± 3.79	56.41 ± 5.11
		Median	53.88	55.39
		Min-Max	51.13-62.93	49.91-64.72

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Table 9. Time of preparation in seconds and number of pecking movements with ProTaper Gold® (Dentsply-Sirona) of 3D printed teeth

3D Printed Teeth		Time of Preparation	Number of pecking movements
ProGlyder®	Mean ± SD	27.30 ± 3.86	14.70 ± 6.24
	Median	27.00	18.00
	Min-Max	21-33	6-21
ProTaper Gold® Sx	Mean ± SD	25.80 ± 3.80	15.00 ± 7.07
	Median	25.50	16.50
	Min-Max	21-33	6-27
ProTaper Gold® S1	Mean ± SD	32.70 ± 4.35	15.30 ± 4.35
	Median	33.00	18.00
	Min-Max	27-39	9-18
ProTaper Gold® S2	Mean ± SD	32.10 ± 2.47	17.10 ± 3.76
	Median	31.50	18.00
	Min-Max	30-36	12-21
ProTaper Gold® F1	Mean ± SD	27.90 ± 4.25	13.20 ± 5.69
	Median	27.00	13.50
	Min-Max	24-36	6-21
ProTaper Gold® F2	Mean ± SD	31.20 ± 5.14	17.70 ± 8.30
	Median	30.00	22.50
	Min-Max	21-39	6-24
Total	Mean ± SD	177.00 ± 8.72	93.00 ± 34.41
	Median	175.50	103.50
	Min-Max	165-195	45-132

Table 10. Time of preparation in seconds and number of pecking movements with Endogal® (Endogal, Galician Endodontics Company) of 3D printed teeth

3D Printed Teeth		Time of Preparation	Number of pecking movements
Endogal® A	Mean ± SD	6.90 ± 1.45	5.10 ± 1.45
	Median	6.00	6.00
	Min-Max	6-9	3-6
Endogal® X	Mean ± SD	27.60 ± 3.69	9.30 ± 3.30
	Median	27.00	9.00
	Min-Max	24-33	6-15
Endogal® B	Mean ± SD	18.00 ± 3.16	8.40 ± 1.90
	Median	18.00	9.00
	Min-Max	15-24	6-12
Endogal® C	Mean ± SD	21.00 ± 4.00	10.50 ± 1.58
	Median	21.00	10.50
	Min-Max	15-27	9-12
Endogal®) D	Mean ± SD	38.10 ± 6.17	14.10 ± 1.45
	Median	39.00	15.00
	Min-Max	27-48	12-15
Total	Mean ± SD	111.60 ± 6.90	47.40 ± 3.10
	Median	111.00	46.50
	Min-Max	96-120	45-54

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6. DISCUSSION

Sections of this chapter has already been published as:

Reis, T., et al., Root Canal Preparation of a Commercial Artificial Tooth versus Natural Tooth - A MicroCT Study. Applied Sciences, 2023. 13(16): p. 9400.
https://doi.org/10.3390/app13169400

Reis, T., et al., Three-Dimensional Printed Teeth in Endodontics: A New Protocol for Microcomputed Tomography Studies. Materials. 2024; 17(8):1899.
https://doi.org/10.3390/ma17081899

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6. DISCUSSION

6.1 DISCUSSION OF FIRST PRIMARY AIM

The first primary aim of this study was to assess how the support material of the TrueTooth® (DELabs) replica influences the preparation technique and its potential impact on volume changes evaluated through micro-CT scans. The findings revealed a negative mean value for root canal volume increase post-preparation in mesiobuccal canal, indicating a loss of volume after preparation, which appears absurd. It was also confirmed with the distobuccal canal that although it had a positive mean value, it still exhibited negative values in some samples for volume increase.

Consequently, it is evident that the support material significantly affects the 3D analysis. Hence, the null hypothesis was rejected. As depicted in Figure 6 and Figure 7, the density of the support material post-preparation exceeded its pre-preparation density and approached that of the resin composing the root. Consequently, following canal preparation, the 3D analysis software no longer distinguishes these materials as separate entities, whereas such differentiation was feasible prior to preparation.

To the best of the author's knowledge, no prior studies have investigated this aspect using micro-CT, thus preventing direct comparison of our findings with existing literature. Our hypothesis suggests that density alterations might result from the heat generated by rotary files rubbing against the canal walls, causing the support material to compress against the areas untouched by the files. Such frictional heat phenomena have been documented and utilized in non-surgical endodontic retreatments (Betti & Bramante, 2001).

The mesiobuccal canal is a large ribbon-shaped canal. Studies have indicated that oval and elongated oval root canals pose challenges during preparation. This difficulty arises from the tendency of files to stay centralized within the canal, creating a rounded preparation, leaving recesses untouched at the ends of the oval canal's largest diameter (Aazzouzi-Raiss et al., 2023; Arias & Peters, 2022; Lacerda et al., 2017; Paque et al., 2010; Wu et al., 2000). Canals with this morphology display a significant amount of unprepared areas, ranging from 10% to 82%, compared to small or round canals which exhibit between 10% to 50%, this could explain the difference of behaviour between artificial mesiobuccal and artificial distobuccal (Siqueira Junior et al., 2018).

In this way, TrueTooth® (DELabs) cannot be used in preparation studies analysed by Micro-CT, due to incomplete removal of the support material by sodium hypochlorite as recommended by the developer (Buchanan, 2014). Future studies with different support material removal protocol are needed.

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However, the mean increase in root canal volume in the natural teeth group was 1.35 mm^3, which aligns with findings from prior studies indicating root canal volume increases ranging from 1.33 to 2.77 mm^3 using the ProTaper Gold® system (Dentsply Sirona)

system (Duque et al., 2017; Gagliardi et al., 2015; Martins et al., 2022; Silva et al., 2021). Nevertheless, all these studies were conducted in mesial canals of mandibular molars. It has been demonstrated that discrepancies in canal geometry prior to preparation may exert a more significant influence on the observed alterations than the preparation techniques alone (Peters et al., 2001). This could explain the disparities among study findings.

The secondary aim was to evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system, on TrueTooth® (DELabs) compared to natural teeth. The mean time of preparation for artificial teeth was one second less, and fewer pecking movements were required for canal preparation compared to natural teeth. However, there were no statistically significant differences between them. Hence, the null hypothesis was accepted. These findings suggest that despite artificial teeth requiring less time and fewer pecking movements for preparation, possibly due to variations in hardness between resin and human dentin (Al-Sudani & Basudan, 2017; Hulsmann, 2022; Reymus et al., 2020), no statistically significant differences were observed. Consequently, the null hypothesis was accepted. This outcome aligns with the findings of Luz et al. (2015), who compared preparation times between natural and artificial teeth using hand-files among students and specialists. Their study concluded that the preparation time for artificial teeth was consistently shorter than for natural teeth across both groups (Luz et al., 2015).

The average preparation time for natural teeth using the ProTaper Gold® (Dentsply-Sirona) system was recorded at 64.3 seconds. This duration is shorter compared to the findings of Arslan et al. (2017), in which the mean preparation time was 78 seconds, however, the absence of information regarding whether the glyde path was established manually or mechanically, or whether it was established at all, was not addressed by the authors (Arslan et al., 2017). This discrepancy between the two studies' results could potentially be attributed to methodological differences. Literature suggests that a mechanically established glyde path tends to be swifter than a manual one (Paleker & van der Vyver, 2017; Zheng et al., 2018). Additionally, if a glyde path is omitted, the root preparation duration is likely to be prolonged (Kirici et al., 2020).

Yamada et al. (2023) documented a preparation time of 43.46 seconds when dealing with "J" canals in resin clear blocks using the ProTaper Gold® (Dentsply-Sirona) (Yamada et al., 2023). Nevertheless, the time needed for preparing this canal type is notably shorter compared to both natural and artificial teeth (Luz et al., 2015).

In Bitter et al. study (2016), 43 students were divided into two groups: one group trained exclusively on TrueTooth® (the test group), while the other group trained on natural teeth (the control group). Following a training period, both groups prepared a natural tooth and an artificial tooth, and their performance was assessed. The study concluded that students exclusively trained on artificial teeth did not demonstrate significantly different performance on natural teeth compared to those trained on natural teeth. However, overall, students tended to exhibit better performance on artificial teeth than on natural teeth (Bitter et al., 2016).

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Moreover, in Gancedo-Caravia et al. study (2020), students were tasked with conducting endodontic treatments on various commercially available artificial teeth, and their performance was subsequently evaluated by three proficient endodontic educators. The evaluation of artificial teeth encompassed three viewpoints: students' achievements,

students' perceptions, and educators' perceptions. The educators concluded that replicas derived from microCT scans more accurately replicated internal anatomy, with TrueTooth® (DELabs) specifically identified as the optimal replica for training in access cavity preparation and negotiation. Conversely, students assessed and performed less proficiently on TrueTooth® (DELabs) likely due to its higher level of difficulty compared to artificial teeth (Gancedo-Caravia et al., 2020).

The findings from these studies, along with those from the current research concerning preparation time and the number of pecking movements required for root canal preparation, suggest that TrueTooth® (DELabs) holds promise for endodontic training. Nonetheless, further investigations employing different preparation systems, diverse geometries, and different kinematics preparation movements are needed in the future.

6.2 DISCUSSION OF SECOND PRIMARY AIM

The sencond primary aim was to describe a support material removal protocol and to compare root canal anatomy between a natural tooth and 3D printed teeth using Micro-CT scans of the natural tooth as a reference. Micro-CT, recognized for its non-invasive and non-destructive nature, enables the assessment of various parameters both pre- and post-preparation. It is widely regarded as the gold standard in root canal preparation research (Hulsmann, 2022).

Upon comparing the volume and centroids X, Y, Z of the natural tooth with those of the 3D printed teeth prior to preparation, our results revealed no statistically significant differences between natural tooth and 3D printed teeth. As a result, the null hypothesis was accepted. These findings indicate that the described support material removal protocol is effective in eliminating the support material (SUP706B™) utilized in this study, and PolyJet® is suitable for producing 3D printed teeth with internal anatomy similar to that of natural teeth.

The support material removal protocol outlined in this study is based on the manufacturer's guidelines for removing the support material SUP706B™ through solubilization. This process involves the use of a 2% alkaline solution of sodium hydroxide and a 1% solution of sodium metasilicate in a cleaning station, followed by rinsing with water (Stratasys, 2019). Therefore, an alternative approach was developed, wherein sodium hydroxide serves as the primary component of the support material removal protocol. Additionally, Derquim® LM 01 alkalin detergent (ITW Reagents, S.R.L.), a general cleaning agent for laboratory use, containing sodium hydroxide (<5%) along with anionic and non-ionic surfactants (15-30%), was employed to facilitate cleaning action on the root canal walls, mimicking the detergent function of sodium metasilicate (ITWreagents, 2016).

Sonic activation with Endoactivator® (Dentsply-Sirona) was employed to enhance the action of irrigants by generating intra-canal agitation and streaming. However, as the Endoactivator® (Dentsply-Sirona) Tip's contact with the root canal walls hinders its free oscillation and diminishes irrigant streaming, the tip was positioned as deeply as possible without making contact with the walls (Donnermeyer et al., 2023; Jiang et al., 2010). However, even in the event of contact, the Endoactivator® (Dentsply-Sirona) Tip, being made of polyamide, does not actively cut the root canal, thereby reducing the risk of altering root canal anatomy (Haupt et al., 2020; Mathew et al., 2023).

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The Irriflex® (Produits dentaires SA) needle utilized is a polyethylene flexible needle, which ensures smoother advancement within the canal without wedging against the root canal walls. It features two side openings that generate jets directed towards the root canal walls, facilitating the delivery of a substantial volume of irrigant at a high flow rate while minimizing the risk of apical extrusion (Provoost et al., 2022). However, for a support material removal protocol to be effective, irrigant extrusion is needed as stated in a previous study (Reymus et al., 2019), so irrigation must be carried out with high pressure and the needle tip positioned as close to the apex as posible.

Nevertheless, the results show differences in the values of 3D printed teeth before preparation, indicating that these teeth are not uniform across all specimens. This can be a result of our protocol; where canal patency plays a crucial role in its efficacy, as evidenced by the visual observation of irrigant extrusion mentioned earlier. To ensure canal patency, a K-file #15 was selected for this purpose, given the challenges in removing support material in channels under 150µm (Macdonald et al., 2017), and it was required that the K-file be used five times for each canal. Following this designated number of uses, if patency was compromised, the K-file #15 was employed to restore it. Despite its passive utilization, this implies that the number of insertions of the K-file #15 varied among the canals and its effects on root canal anatomy should be considered (Hartmann et al., 2018; Smutny et al., 2022).

Another concern that warrants attention is that, while PolyJet® printing technology has the smallest dimensional error among various 3D printing technologies (Herpel et al., 2021; Kim et al., 2022; Kim et al., 2018; Rouze l'Alzit et al., 2022), its accuracy is contingent upon factors such as the material utilized, the geometry of the printed object, and the orientation relative to both the printer head and printer bed (Bezek et al., 2016; Kent et al., 2017; Mendřický, 2016; Rouze l'Alzit et al., 2022).

In a prior investigation, a rectangular flat part was printed with its long axis aligned parallel to either the X, Y, or Z axis of the printer bed. It was determined that for the strongest parts, the long axis of the printed part should be parallel to the X axis (Bezek et al., 2016). However, to the best of the author’s knowledge, this aspect has not been explored in the context of printing teeth.

In the case of a single-root tooth, determining the long axis is straightforward, it aligns with the long axis of the root. However, in our study involving multi-rooted teeth, we measured the tooth as a whole, and the longest distance was observed in the buccal to palatal direction. Therefore, this was considered the long axis, and the 3D printed teeth were produced accordingly, as illustrated in Figure 3.

Nevertheless, future studies should assess which is the best orientation to give a multiple root tooth according to the X, Y or Z axis and determine if this orientation significantly impacts the accuracy of 3D printed teeth.

Furthermore, it has been documented that alterations in volume occur during PolyJet® printing due to expansion (Mendřický, 2016; Rebong et al., 2018). The results demonstrate a volume expansion of 0.77%, which could also account for the differences observed among 3D printed teeth. This finding aligns closely with the 0.71% expansion reported in a prior study (Lee et al., 2015).

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Additionally, it's worth noting that the STL file underwent simplification and preparation for 3D printing, which may have led to some degree of distortion (Lee et al., 2015). Future studies should assess the extent of simplification that STL files can undergo without causing significant distortion relatively to natural tooth.

The natural tooth used had its crown sectioned and presented an internal anatomy with three single different types of canals, however it did not present any anatomical irregularities such as lateral canals or sthmuses, which are known to be challenging areas for irrigants' access (Chan et al., 2023), future studies are needed to evaluate the effectiveness of the support material in teeth with full crown and with anatomical irregularities.

Regarding the first secondary aim, in order 3D printed teeth to be used in ex vivo preparation studies, its behaviour to preparation should be similar to natural tooth, the present results regarding volume of dentin removed, percentage of volume increase, transportation, and unprepared areas, show that there were no statistically significant differences between them. Hence, the null hypothesis was accepted, leading to the conclusion that 3D printed teeth behave similarly to natural tooth when prepared with ProTaper Gold® (Dentsply-Sirona).

Regarding the second secondary aim, to evaluate the time and number of pecking movements required for root canal preparation using the ProTaper Gold® (Dentsply-Sirona) system on natural tooth compared to 3D printed teeth, it was showed that 3D printed teeth required less preparation time and less pecking movements. However, there were no statistically significant differences between them.

These findings align with our earlier research conducted on the preparation of TrueTooth® and are consistent with Luz et al. (2015) observations (Luz et al., 2015; Reis et al., 2023), that less time and fewer pecking movements are necessary for preparing tooth replicas compared to natural teeth, nonetheless without statistically significant differences between them. Hence, the null hypothesis was accepted, reinforcing the conclusion of the first secondary aim, that 3D printed teeth behave similarly to natural tooth when prepared with ProTaper Gold® (Dentsply-Sirona).

The major concern raised regarding 3D printed teeth is the difference in hardness between resin and human dentin (Hulsmann, 2022; Karatekin et al., 2019; Kolling et al., 2021; Ordinola-Zapata et al., 2014; Reymus et al., 2019). The High Temperature RGD525™ is an opaque model material that has exceptional dimensional stability and presents a tensile strength ranging from 70 to 80 MPa, a modulus of elasticity ranging from 3200 to 3500 MPa, a flexural strength ranging from 110 to 130 MPa (Stratasys, 2022). In contrast, human dentin exhibits a tensile strength ranging from 44.4 to 97.8 MPa, with the lower value typically found in the inner dentin near the pulp (Staninec et al., 2002). Its modulus of elasticity falls between 1375 and 1931 MPa (Chun et al., 2014), and its flexural strength ranges from 171 to 254 MPa (Plotino et al., 2007). While the printing material and dentin exhibit comparable values, disparities in their structural organization persist due to dentin's tubular nature. Consequently, there is a necessity for the advancement of both printing materials and techniques to replicate the natural structure of teeth in 3D printed teeth.

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Regarding the third secondary aim, to assess the behaviour of 3D printed teeth to preparation with ProTaper Gold® (Dentsply-Sirona) up to F2 file and Endogal® (Endogal, Galician endodontics Company) up to D file. This involved comparing volume increase, volume of dentin removed, centroids, transportation, unprepared areas using micro-CT scans. Results of volume of dentin removed, percentage of volume increase, transportation, and unprepared areas, show that there were no statistically significant differences between the two preparation systems. Thus, the null hypothesis was accepted.

However, upon examining the results individually, it becomes evident that Endogal® (Endogal, Galician Endodontics Company) resulted in less volume increase and dentin removal, along with a higher percentage of unprepared areas. Despite both systems having the same apical size for preparation, variances in taper and cross-section design could account for these differences. Differences in root canal preparation are also associated with the dimensions of the files utilized (Chan et al., 2023; Silva et al., 2020).

To the author's knowledge there is no other study that evaluate the shaping properties of Endogal® (Endogal, Galician Endodontics Company) system, so the present results cannot be compared to others.

Regarding the results observed to ProTaper Gold® (Dentsply-Sirona) system in the present study, the percentage of volume increase was 47,68%, while in the literature, it ranges from 18.7% to 163.32% (Aazzouzi-Raiss et al., 2023; Duque et al., 2017; Gagliardi et al., 2015; Silva et al., 2023; Sivakumar et al., 2023). The percentage of unprepared area was 54.97%, in literature ranges from 3.57% to 46.85% (Aazzouzi-Raiss et al., 2023; Duque et al., 2017; Gagliardi et al., 2015; Martins et al., 2022; Nehme et al., 2021; Silva et al., 2023; Silva et al., 2020; Sivakumar et al., 2023; Yalniz et al., 2021).

The differences between our results and literature can be attributed to the types of teeth or canals examined. Many studies predominantly focus on mandibular molar mesial canals, which tend to exhibit higher values of percentage volume increase and smaller values of percentage unprepared area (Duque et al., 2017; Gagliardi et al., 2015; Silva et al., 2023; Siqueira Junior et al., 2018; Sivakumar et al., 2023). The disparities between the results of our study and those in the literature can be attributed to the specific types of teeth or canals used. Many studies focus on mandibular molar mesial canals, which typically exhibit higher values of percentage volume increase and smaller values of percentage unprepared area (Duque et al., 2017; Gagliardi et al., 2015; Silva et al., 2023; Sivakumar et al., 2023). Conversely, studies involving mandibular incisors or premolars may yield different outcomes (Aazzouzi-Raiss et al., 2023; Yalniz et al., 2021). Variations in canal geometry can significantly influence preparation techniques. It is widely acknowledged that shaping oval-shaped canals presents a challenge, and smaller values of percentage unprepared area in such canals are linked to brushing movements during preparation rather than pecking movements (Hilaly Eid & Wanees Amin, 2011; Romeiro et al., 2023).

The transportation was 0.48 mm; however, comparing root canal transportation can be challenging due to non-standardized methods. It can be assessed by measuring the distance of dentin removed, typically measured at three arbitrary points or the center point of the canal in all slices of the stack. Alternatively, a simplified measurement involves assessing the displacement of the canal center point in 3D (Fidler et al., 2021; Serefoglu & Piskin, 2017; Ventura et al., 2024). The methodology employed in this study provides

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deviation in all directions, which may yield higher values compared to other methodologies. However, despite this, it appears to be the most suitable and straightforward to interpret (Fidler et al., 2021; Ventura et al., 2024). To the best of the author's knowledge, no previous study has utilized this methodology with respect to ProTaper Gold® (Dentsply-Sirona), so it this result cannot be compared with others.

All preparations were conducted by an experienced dentist in the field of endodontics with clinical experience with the two systems. The primary criticism raised by the operator was a greater screw-in effect observed in 3D printed teeth compared to natural teeth, resulting in more challenging preparations. This effect refers to the tendency of a rotary file to be drawn into the canal, influenced by factors such as movement kinematics, file cross-section, taper, and rotational speed (Ha et al., 2017; Ha & Park, 2012; Kimura et al., 2020; Kyaw et al., 2021; Wang et al., 2022). The manufacturer's recommendations of rotation per minute and torque were used, future studies are needed to establish how changes in rotations per minute, torque, pecking movements amplitude values and files design can influence 3D printed teeth preparation.

Concerning the fourth secondary aim, which assess the time and number of pecking movements necessary for root canal preparation on 3D printed teeth using the ProTaper Gold® (Dentsply-Sirona) system compared to Endogal® (Endogal, Galician Endodontics Company) system, there was statistically significant differences between them, being the Endogal® (Endogal, Galician Endodontics Company) faster and requiring fewer pecking movements. In this way the null hypothesis was rejected. However, these findings are straightforward to interpret and expected, as the Endogal® (Endogal, Galician Endodontics Company) system utilizes one less file compared to the ProTaper Gold® (Dentsply-Sirona) system.

Also, statistically significant differences were observed between the ProGlyder® file (Dentsply Sirona) and A file (Endogal, Galician Endodontics Company). The preparation time recorded was 27.30 seconds for the ProGlyder® (Dentsply Sirona) and 6.90 seconds for the A file (Endogal, Galician Endodontics Company), while the pecking movements were 14.70 and 5.10 respectively.

As previously noted, the ProGlyder® file (Dentsply Sirona) is characterized by a tip size of 0.16 mm and a variable taper ranging between 2% and 8.5% (Alovisi et al., 2017; Gambarini et al., 2021; Keskin et al., 2020; Kirchhoff et al., 2015; Paleker & van der Vyver, 2017). In contrast, the A file (Endogal, Galician Endodontics Company) maintains a consistent 3% taper with a tip size of 0.15 mm (Castelo-Baz et al., 2023; Endogal; Faus-Matoses et al., 2022; Ruiz-Pinon et al., 2021). Although both files share a similar apical size, differences in taper and cross-section design may explain these diferences.

Another noteworthy aspect is the use of a K-File #15 during the support material removal protocol. Although it was employed passively, its potential impact on root canal anatomy should be acknowledged (Hartmann et al., 2018; Smutny et al., 2022), potentially facilitating the action of the A file (Endogal, Galician Endodontics Company).

No other study has assessed the preparation time and pecking movements required by the A File (Endogal, Galician Endodontics Company). Therefore, the findings of this study cannot be compared to those of others.

In relation to the results observed with the ProGlyder® file (Dentsply Sirona), the preparation time for the three root canals was 27.30 seconds with 14.70 pecking movements. In existing literature, the preparation time ranges from 6.55 to 14.87 seconds for a single root canal (Gambarini et al., 2021; Yamada et al., 2023), and 67.40 seconds for three root canals (Dablanca‐Blanco et al., 2021). However, the methodologies used for time measurements in these studies lack clarity, making comparison with our results challenging. Kirchhoff et al. (2015) and Alovisi et al (2017) employed a methodology similar to this study and reported a preparation time of 7.38 and 6.8 seconds, respectively, for a single root canal (Alovisi et al., 2017; Kirchhoff et al., 2015).

The pecking movements required was 14.70 for three root canals, contrasting with the literature, which indicates a requirement of 3.80 pecking movements for a single root canal (Alovisi et al., 2017).

As said before, protocols for standardization of studies using 3D printed teeth still have to be developed (Hulsmann, 2022), our results within its limitations presents a description of an effective protocol for support material SUP706B™ removal, demonstrate that 3D printed teeth printed with High Temperature RGD525™ material have similar behaviour to endodontic preparation that natural teeth.

However, future research is necessary to standardize studies involving 3D printed teeth. This entails determining the optimal orientation on the printer bed for multi-rooted teeth, deciding the level of simplification for the STL file without sacrificing information, assessing the effectiveness of support material removal protocol for various root morphologies, and understanding how variations in rpm, torque, amplitude of pecking movements, and file design can impact the preparation of 3D printed teeth. Ultimatley when a solid protocol has been developed for preparation studies, future studies should address the possibility of using 3D printed teeth in irrigation and obturation studies, aiming to create definitive model for endodontic studies. Aditionally, another perspective that future researchs should address is to investigate the impact of training with 3D printed teeth on clinical outcomes and their effectiveness in enhancing endodontic skills. This would provide valuable evidence for their incorporation on daily basis into endodontic education.

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7. CONCLUSIONS

Sections of this chapter has already been published as:

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7. CONCLUSIONS

1. In the 3D analysis of the artificial teeth's mesiobuccal canal, the average increase in root canal volume was found to be negative, indicating that the support material influenced the Micro-CT 3D analysis of the variable under consideration.

2. No statistically significant differences were found in the time and number of pecking movements between natural teeth and artificial teeth. Thus, it can be concluded that TrueTooth® serves as a suitable replica with potential application in endodontic training.

3. The described support material removal protocol can be concluded as effective in eliminating the support material from 3D printed teeth root canals.

4. The PolyJet™ technique is suitable for producing 3D printed teeth.

5. 3D printed teeth produced with High Temperature RGD525™ have similar behaviour to endodontic preparation that natural tooth, and 3D printed teeth can be used when comparing two preparation systems.

   The potential exists for 3D printed teeth to establish a new standard in ex vivo studies and endodontic training, potentially supplanting natural teeth. However, achieving this requires further research to address the current limitations of 3D printed teeth. Additionally, there is a need for concerted efforts to establish robust protocols, facilitating method standardization and enabling comparison of results across studies.

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11. APPENDICES

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11. APPENDICES

APPENDIX 1 - APPROVAL BY THE ETHICS COMMITTEE OF INSTITUTO ASTURIANO DE ODONTOLOGIA OF OVIEDO UNIVERSITY (IAO-23-0104)

Universidad de Oviedo

Instituto Asturiano de Odontología

A quién corresponda

El Comité de Ética del Instituto Asturiano de Odontología (IAO), en su reunión del 22 de mayo de 2023, aprobó el estudio titulado **"Root canal preparation of a comercial artifical tooth versus natural tooth – a Micro – CT study"**, con la Referencia Nº: IAO-23-0104. Supervisado por el Prof. Benjamín Martín Biedma.

En Oviedo, 22 de mayo de 2023

Universidad de Oviedo

Prof. Juan Cobo Plana
Catedrático de Universidad
Presidente del Comité

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Dña. Sara Álvarez Suárez
Coordinadora de Calidad del IAO
Secretaria del Comité

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APPENDIX 2 - APPROVAL BY THE ETHICS COMMITTEE OF FERNANDO PESSOA UNIVERSITY (FCS/PI 429/23)

UNIVERSIDADE FERNANDO PESSOA

Exma. Senhora
Prof. Doutora Sandra Gavinha
Diretora da FCS

Nº	Data
FCS/PI – 429/23	30 de Junho de 2023

Exmª. Senhora Professora Doutora,

A Comissão de Ética apreciou a resubmissão do projeto de investigação, apresentado pelo Professor Doutor Tiago Reis, intitulado "3D printed teeth in endodontics: a new protocol for Micro-CT studies".

A Comissão de Ética considera o estudo pertinente e interessante e com potencial de aplicação futura.

Todas as explicações solicitadas no parecer anterior foram dadas. Deste modo, a Comissão de Ética nada tem a opor quanto à realização deste estudo.

Com os melhores cumprimentos,

A Presidente da
Comissão de Ética da UFP
Inês Lopes Cardoso

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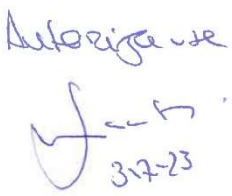

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PUBLICATION DERIVED FROM THIS THESIS

APPENDIX 3 – PERMISSION TO REPRODUCE PAPER

Title: 3D Printed Teeth in Endodontics: Why, How, Problems and Future - A Narrative Review
Authors: Tiago Reis[1,2,*], Cláudia Barbosa[3], Margarida Franco[4], Catarina Baptista[4], Nuno Alves[4], Pablo Castelo-Baz[1], José Martin-Cruces[1] and Benjamin Martin-Biedma[1]
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Review

3D-Printed Teeth in Endodontics: Why, How, Problems and Future—A Narrative Review

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Abstract: Three-dimensional printing offers possibilities for the development of new models in endodontics. Numerous studies have used 3D-printed teeth; however, protocols for the standardization of studies still need to be developed. Another problem with 3D-printed teeth is the different areas of literature requested to understand the processes. This review aims to gather evidence about 3D-printed teeth on the following aspects: (1) why they are advantageous; (2) how they are manufactured; (3) problems they present; and (4) future research topics. Natural teeth are still the standard practice in ex vivo studies and pre-clinical courses, but they have several drawbacks. Printed teeth may overcome all limitations of natural teeth. Printing technology relies on 3D data and post-processing tools to form a 3D model, ultimately generating a prototype using 3D printers. The major concerns with 3D-printed teeth are the resin hardness and printing accuracy of the canal anatomy. Guidance is presented for future studies to solve the problems of 3D-printed teeth and develop well-established protocols, for the standardization of methods to be achieved. In the future, 3D-printed teeth have the possibility to become the gold standard in ex vivo studies and endodontic training.

Keywords: endodontics; printing; three dimensional; tooth; 3D-printed teeth; review

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1. Introduction

Bacteria are the main cause of pulpal and periapical pathologies. Therefore, all procedures used in endodontics are aimed at eliminating pulp tissue as well as many bacteria as possible [1,2].

The American Association of Endodontists defines root canal preparation as "Procedures involved in cleaning and shaping the canal system prior to obturation", distinguishing between "biomechanical preparation" as the "use of rotary/reciprocating and/or hand instruments to expose, clean, enlarge and shape the pulp canal space, usually in conjunction with irrigants" and "chemomechanical preparation" as the "use of chemicals for irrigation of the root canal, demineralization of dentin, dissolution of pulp tissue and neutralization of bacterial products and toxins; used in conjunction with biomechanical preparation" [3].

To achieve these goals, a variety of manual rotary instruments have been proposed. The revolution in the manufacturing root canal instruments leads to a great variety of these endodontic instruments when comparing the design, alloys and type of movement [2,4–8].

Adequate knowledge of the shaping properties and performance of rotary or reciprocating instruments is essential to assist the clinician in selecting the appropriate system for

different clinical situations, as new instrumentation systems continue to be introduced into the market [9,10].

For a successful root canal treatment, the knowledge of root canal anatomy is a basic requirement [11]. The general root canal anatomy of a normal tooth and other anatomic variations have been identified in many morphologic studies [11–13]; however, the anatomy of the root canal can vary widely at different levels of the root [14].

Teaching root canal treatment is challenging in undergraduate and postgraduate education. European Society of Endodontology guidelines state that students should be suitably trained to perform good-quality root canal treatment [15,16]. Nonetheless, a recent study stated that Spanish dental students treated simple clinical cases in 100% of the schools, but moderate clinical cases only in 40%, and only 60% of schools have minimum requirements for the number of root canal treatment cases [17]. Furthermore, in another study, a high proportion of final-year dental students were not confident in performing root canal treatment on multirooted posterior teeth [18]. Thus, in dental education, there is a need for hands-on pre-clinical training before a patient's treatment is carried out for the first time [16,19–22].

Traditionally, for ex vivo studies and pre-clinical courses, extracted human teeth have been the standard practice [23–28] and are considered the best for simulating the clinical setting, despite the lack of evidence to support this claim [29], providing a good understanding of the internal anatomy and allowing students to experience the tactile sensation of working on dentine [30].

However, extracted human teeth has several drawbacks, which are being discussed in recent years: they are difficult to collect, there are ethical considerations, potential cross-infection risks and storage drawbacks and standardization issues [19,23,26,31–33].

Artificial teeth that reproduce the features of natural teeth may overcome these limitations [19,28,31,34]. In recent years, different factory-manufactured models have been introduced in the market, which are realistic and standardized. However, their costs are high, there is a limited selection of tooth types, the delivery times must be considered with a dependency on a manufacturer and the differences between the manufacturing process and the material used by each commercial brand are widely different [23,26,31,35].

Three-dimensional printing is a rapidly developing technology that has gained widespread acceptance in dentistry [36], and with costs constantly declining and more materials available, 3D-printed teeth offer unexpected possibilities for the development of new, individual models that are not yet available on the market or are too expensive to purchase in high quantities [26,28,31,34]. Nonetheless, the major criticism of 3D-printed teeth is the difference in radiopacity and hardness between resin and human dentine [19,23,31].

Numerous studies are using 3D-printed teeth; however, protocols for the standardization of studies still need to be developed since there exists a great methodological variation between these studies [23,24].

Another problem with 3D-printed teeth is the different and scattered areas of the literature, which need to be mastered to understand all the processes of printing them.

Hence, the aim of this review is to gather evidence on 3D-printed teeth on the following aspects: (1) why they are advantageous, pointing out the problems with natural teeth that they overcome; (2) how they are manufactured, describing all the phases and options of printing; (3) the problems and disadvantages that they present; and (4) future research topics to overcome these problems.

For the purposes of the present review, a search was conducted in PubMed, MEDLINE, Scopus, Web of Science, until 16 April 2022, using the keywords Endodontics, 3D printed teeth, artificial teeth, 3D printing, dentin, teaching, alone and with the Boolean operator "AND" in different associations. Original research studies, review articles and case reports, in English, without time limitation, were selected if relevant to the aims of this review. Additionally, cross references were used.

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2. Why

Natural teeth are still the standard practice in ex vivo studies and pre-clinical courses [23–28]. Their advantages include natural tissue hardness, morphology, color, texture and radiodensity [24,28], but as listed before, they have several drawbacks:

(1) Difficult to collect: Increasing challenges in sourcing sufficient suitable natural teeth, probably as a result of improvement in health standards; it is time-consuming and requires a large number of teeth (wisdom teeth, teeth with highly complex root canal morphology and with previous root canal treatment are not acceptable, and natural teeth must present complete root development, an intact crown and intact roots) [19,23,24,27,30,35,37]. Nowadays, the biobank is considered a primary resource for the development of precision medicine. The purpose of biobanks is to support and improve clinical and biomedical research, especially for preclinical trials [38,39]. As a result, these teeth are not available for teaching purposes.

(2) Ethical considerations: Previous owners of natural teeth, representing biomaterial, should have declared their consent to the use of their teeth [23,28,32]. A worrying phenomenon is that in buying human teeth online, pressures to source teeth are likely to encourage investigators and students not to question where such teeth are from and why they have been extracted [32].

(3) Potential cross-infection risk and storage: Natural teeth are grossly contaminated, difficult to sterilize and common disinfection procedures, such as using sodium hypochlorite and hydrogen peroxide, are not effective [40,41]; teeth could also be damaged or altered by the sterilization and storage procedures. In a study that investigated the effect of disinfection methods on tooth resistance to fracture, it was found that autoclaving teeth reduced their resistance to fracture [42]. Another study investigated the effect of storage conditions on tooth resistance to fracture, and it was found that teeth stored in water have a higher resistance to fracture than teeth stored dry [43], so the preparation of natural teeth is an unpleasant task as they must be stored in a strong-smelling liquid [44].

(4) Standardization: The difficulty of creating well-balanced groups in ex vivo studies and an equal student assessment is a consequence of the intricate anatomy of the root canal system [14,25,26,31,33,45]. As a consequence, studies might demonstrate the effect of canal anatomy rather than the variable of interest [33].

Students normally comment that the anatomical variability does not allow a valid assessment of their individual performance [37].

The most common method used in ex vivo studies is to create pair-matched tooth samples according to their anatomical features, such as the tooth type, root length, root anatomy, degree and angle of curvature and root canal diameter, or even with paired contralateral teeth. Some studies allocated teeth from the same group by randomization with or without radiographs [23,25,46].

Recently, some studies started to use specific anatomical parameters identified by means of a microcomputed tomography (micro-CT) scanning [23,25]. De-Deus et al. 2020 compared the randomized method, radiographic method and micro-CT method. It was concluded that the micro-CT method was able to provide better control of the anatomical variance confounding effect. Nonetheless, this study was carried out from an initial pool of 1708 single-rooted mandibular incisors, and for the group of the micro-CT method, 251 teeth were chosen, which were scanned, to create two anatomically pair-matched groups (n = 10) [25], demonstrating the difficult, time-consuming and high-cost nature of this method.

Additionally, it should be noted that other tooth parameters should be taken into count, which cannot be sufficiently standardized by these methods, such as the age and ethnicity of donor and environmental factors. These factors have an impact on the mechanical properties of dentin [23,47,48].

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Dentin is a hard tissue that occupies the majority of the human tooth, which possesses a complicated hierarchical structure. It consists of approximately 45% mineral material, 33% organic material and 22% water [48,49].

The microstructure of dentin is largely dominated by its tubules, which extend radially outward from the pulp towards the dentin–enamel junction. The lumen of each tubule is surrounded by a cuff of peritubular dentin, which consists of a highly mineralized region of apatite crystals and a small proportion of organic proteins. The tissue located between the tubules is called intertubular dentin and contains a matrix of collagen fibers reinforced by apatite [49,50].

After the third decade of life, there is a transition in the microstructure of dentin, in which the inorganic material increases and tubules become gradually filled with it [48–51], consequently diminishing the tubule density and dentinal diameter. These modifications produce variations in its mechanical properties, such as an increase in elastic modulus and hardness and a decrease in strength and fatigue crack resistance [49–53]. It has been well established that the aging of dentin starts from root dentin and continues in the coronal direction [49,54]. The occlusion rate of dentinal tubules is greatest at the root apex and closer to the pulp in older donors, when compared with younger donors, where it is greater near the cementum [54].

Additionally, in a study of the microstructure of dentin and its mechanical behavior, existing differences were demonstrated between the dentin of donors with an origin in the United States of America from those with an origin in Colombia [47].

For the reasons listed above, a total standardization is, with actual methods, almost impossible to achieve.

Three-dimensional-printed teeth that reproduce the features of natural teeth may overcome all these limitations [19,28,31,34] and are also suitable for practicing access opening, canal instrumentation, radiographic length control and canal filling [26,28].

(1) Difficult to collect: There is no problem in collecting them since the selection of different teeth is not limited and they are available immediately in a sufficient number [19,23,26,28,44]. Additionally, an online platform where institutions could share their printable files would be a considerable benefit for the entire community [26].
(2) Ethical considerations: Only 3D-printed teeth generated from cone beam computer tomography (CBCT) or micro-CT of natural teeth should require donor consent for the use of their teeth [23,28,32]; however, the number of natural teeth needed would be diminutive.
(3) Potential cross-infection risk and storage: They present no risk, are more hygienic and have better handling since they do not need to be stored in liquid, providing a safe training environment [19,44,55].
(4) Standardization: They are realistic and standardized, so the same level of difficulty is guaranteed for every single student and, consequently, they will be scored fairly [19,26–28,44,56], because this enables the definition and standardization of specific grading criteria [30,57]. This standardization allows students and instructors to focus on learning and teaching the clinical procedures, rather than dealing with the morphological variability of natural teeth [24]. Additionally, in ex vivo studies, this morphological standardization has a major impact on the credibility of results [2,25,33,58,59].

Three-dimensional-printed teeth have other advantages in pre-clinical courses such as presenting canal anatomy difficulty in a progressive way [2,19]; students can practice the procedures as many times as they want and even compare different protocols, which is also true for ex vivo studies [2,19,35]. It seems logical that techniques well covered in pre-clinical course are performed more easily and cause less stress among the students when treating patients for the first time [16].

Tchorz et al. 2015 compared the effect of students training with artificial teeth and with extracted human teeth on the quality of the first root canal treatment performed on patients.

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They were unable to detect any differences between the two groups [37], demonstrating that artificial teeth are suitable for endodontic training.

Various studies report that students highly appreciated the use of 3D-printed teeth for the standardization of endodontic training and as a realistic simulation of clinical treatment [26,34,55]. Moreover, instructors appreciated 3D-printed teeth, possibly because they also experience the difficulties of pre-clinical courses on natural teeth [27,30].

Another use for 3D-printed teeth is in complex anatomical clinical cases, allowing clinicians to understand the root canal configuration and try a specific clinical treatment before reproducing it on a patient. The clinician can train and find the best approach to be more confident during the real treatment [1,60,61].

3. How

Additive manufacturing, also known as 3D printing, is the process of joining materials to make parts from 3D model data, usually layer upon layer [62]. This technology relies on 3D data, post-processing tools and algorithms to restructure and edit multiple planes to form a 3D model and ultimately generate a part using a specific material with a 3D printer [1,59,63] (Figure 1).

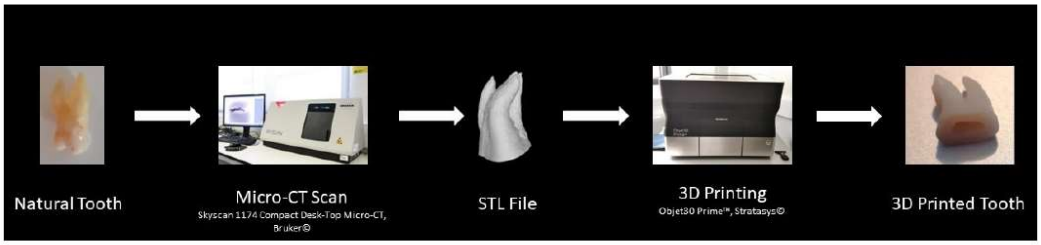

Figure 1. Three-dimensional printing process. (Micro-CT—Micro-Computer Tomography; STL—Standard Tessellation Language).

The first step of 3D printing is to obtain a digital model based on computer tomography (CT) scan data, laser scan data, designed using computer-aided design (CAD) [63,64].

Micro-CT is a non-invasive, non-destructive and high-resolution technology that allows the three-dimensional study of the root canal system. This technology can be used to understand the influence of different procedures on the canals by digitally reconstructing cross-sections of the teeth, which can be grouped to create three-dimensional models [65]. Micro-CT outputs images in Tagged Image File Format (TIFF). These TIFF images are reconstructed in Bitmap (BMP) format and are then managed into 3D construction software to output data in STL format [59,66].

CBCT is a modification of the CT concept, involving the single rotation of an X-ray source around the subject. CBCT outputs images in Digital Imaging and Communications in Medicine (DICOM) format, which are then managed into 3D construction software to output data in STL format [58,63,67].

STL format stands for Standard Tessellation Language or Standard Triangulation Language and represents the virtual 3D surface with the help of triangulation (tessellation). Each triangular face is characterized by its three corner points and the corresponding surface. Curved surfaces are approximated polyhedral. Increasing the number of polyhedral, by diminishing the size of the triangles, describes the object surface with higher resolution; however, this exponentially increases the number of triangles in the file, which results in large file sizes [36,68].

The accuracy of 3D printers is defined by XY resolution and layer thickness, namely the z-axis, which represents the vertical accuracy. The layer thickness typically describes

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the surface finish, so a thinner layer thickness is associated with smoother objects but also with a longer printing time [36,69].

In some 3D printing processes, areas under overhangs, cavities and holes are filled with a support material, and its removal is dependent on the technology used [36].

The various additive processes of major interest for this aim are presented.

Stereolithography (SLA) (layer thickness 25–100 µm) polymerizes by a single ultraviolet (UV) laser, a bath of liquid resin, which is held in a vat [31,36,64,69,70]. A computer-controlled mirror is used to focus the UV laser onto the surface of the resin and cure the resin layer by layer. During the sequential curing process, the layers bind together to form a solid mass, beginning from the bottom of the object and building upward; this is the bottom-up approach [36,69,70]. In contrast, in the top-down approach, the platform can be lowered vertically and is immersed in the reservoir. The resin layer is exposed to a laser that scans from the bottom reservoir [36]. The model is then removed and post-cured for a further period in a UV cabinet for a reduction in the residual resin content [36,70]. The model material is robust, slightly brittle and relatively light [70]. It requires support material [36].

Digital Light Processing (DLP) (layer thickness 25–100 µm) polymerizes by a light source from a conventional DLP projector. Liquid resin is held in a vat [31,36,70]. The projector exposes the resin to a 2D image and the object is produced by layers as the platform is manipulated [69], which is similar to SLA, but there is no moving beam [63]. It requires support material [36].

Fused Deposition Modelling (FDM) (layer thickness 178 or 254 µm) is a method in which layers of molten material are deposited from a filamentous nozzle and then solidify within 0.1 s. Generally, it is less accurate [36,64,69–71]. It may need support material [36].

PolyJet and MultiJet printing technology (layer thickness up to 16 µm) uses photosensitive resin to provide highly accurate product detail, is not limited by geometric shapes and is particularly suitable to simulate root canal anatomy. This method is very similar to the 2D printing process of an inkjet printer [1,36,44,55,64,70]. This technology prints two different materials within a single print job: a model material and the support material [36,44,63,70,71]. The models are created by the jetting of ultra-thin layers of resin that are cured with UV light after each pass onto a build tray that lowers and moves backward and forward. In these technologies, the support material is softer [44,64,69–72]. Typical removal methods include manual breaking, dissolution in water pressure, dissolution in caustic soda or melting by raising the temperature [36,44,72]. These two printing technologies only differ in their support materials and post-processing requirements [36,69,70].

ColorJet printing technology (layer thickness 50–100 µm) is a method in which a print head selectively disperses a binding agent onto layers of powder that are glued together [36,64,69,70]; however, the accuracy, finish and strength are poor [63]. In this method, the support material is not needed because the surrounding powder supports the unconnected parts, and in the finishing process, the prototype is infiltrated with a cyanoacrylate-based material to harden the structure and diminish the porosity [36,70]. It does not require support material [36].

Selective Laser Sintering (SLS) (layer thickness 30–100 µm) and Selective Laser Melting (SLM) (layer thickness 20–100 µm) printers use a computer-directed laser and a roller to distribute layers of powdered material on top of the preceding layer; each new layer is sintered (SLS) or melted (SLM) [36,64,69,70], and they can print a thermoplastic polymer, metal, ceramic or glass [36,63]. They do not require support material [36].

4. Problems

Three-dimensional-printed teeth still present some problems that need to be overcome before they can be used routinely in studies or in education.

Three-dimensional-printed teeth can be used only for studies on the root canal shape; they are not suited for root canal cleanliness or alterations in dentin [23], although, recently, Choi et al. 2021 modified a designed 3D-printed tooth with a slot wherein a dentin segment

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could be inserted. After bacterial contamination of the dentin, various protocols of irrigation were applied and compared [29]; however, more studies are needed with this prototype. Nevertheless, they could be used in obturation studies, Karatekin et al. 2019 and Gok et al. 2017 evaluated different obturation techniques in 3D-printed teeth with C-shape canals, by cross-section at different levels and analyzing the images with magnification [58,73]. In the study by Karatekin et al., 3D-printed teeth were instrumented, and as the authors stated, this could be a limitation of their study, since one major advantage of 3D-printed teeth is standardization and the bias introduced by instrumentation was not considered. In the study by Gok et al., the natural tooth was instrumented prior to the micro-CT scan; this methodology is more adequate for obturation studies. Additionally, Peters et al. 2021 used 3D-printed teeth obtained from a previously prepared tooth to compare the obturation quality between students [74].

It has also been reported that it is difficult to remove the support material from parts with features with fine details [23,26,45,72]. To the author's knowledge, there is no study that demonstrates that the internal root canal anatomy is free of support material, and the majority do not explain the protocol used for removing it from inside the root canals, or even if it was performed [1,55,58,59,66,73]. This way, canals could be filled partially or totally with the support material. The effect of this material on the variables of interest should be considered. Reymus et al. 2019 described the method used in their study for removing the support material. First, on the natural tooth before a CBCT scan, they carried out a retrograde preparation on the apical 5 mm with hand files up to an ISO file 10 to ensure patency, and in sequence, all canals were prepared up to an ISO file 20. Subsequently, the access cavity was closed with a cotton pellet and a radiopaque filling. After being printed, using the SLA technique, the 3D-printed teeth were cleaned with alcohol for 2 min in an ultrasonic bath. Next, they were centrifugated for 10 min at 3500 revolutions per minute to free the canals from the support material [26]. The authors stated that for support material removal, the canal must be patent, and that is the reason for executing the retrograde preparation [26]; however, with this protocol, the internal anatomy of the natural tooth was altered and did not mimic an intricate anatomy. Orel et al., 2021, in a study comparing different instrumentation systems, reported that the apical 3 mm of the canals of 3D-printed teeth, using the SLA technique, were obstructed by the support material, so they manufactured another 3D-printed tooth separated along the long axis of the tooth, into two pieces that were held together with a clamp [45]. Since they were comparing the effects of different instrumentation systems on the canal walls, the fact that the teeth were separated and only held together by a clam during the process could have possible effects on the variables of interest that were not addressed by the authors.

Some studies analyzed the accuracy of 3D-printed teeth by analyzing the external surface [1,26,75], and others, the internal root anatomy [1,59,66].

Reymus et al. 2019 only analyzed the outer surface of the 3D-printed teeth by comparing the STL file of the natural tooth, with STL files of the 3D-printed teeth obtained with a dental scan, and stated that could be assumed that the results would be similar for the internal root anatomy [26], but they did not carry out any evaluation of this parameter.

Liang et al. 2018 compared, by CBCT, the accuracy of the external surface and the internal root anatomy at 3, 6 and 9 mm from the apex, between twenty natural premolars and their respective 3D-printed teeth. It was concluded that 3D-printed teeth did not differ significantly from the natural teeth [1]. It is important to mention that the natural teeth were previously divided into four groups and instrumented previously to 25#06, 30#09, 35#04 and 40#40, respectively, so the results cannot be extrapolated to teeth that would be used in studies or education with intricate canal anatomy.

To the author's knowledge, only one study to date has used micro-CT to compare the internal root anatomy of the natural tooth with the internal root anatomy of 3D-printed teeth [66]. Cui et al. 2018 scanned six pre-molar teeth, three maxillary and three mandibular, and used these scans to construct 3D-printed teeth using the PolyJet technique, removing the support material by melting. However, the protocol was not explained, and it is

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not noted whether the 3D-printed teeth had an access cavity carried out on them. They concluded that the area and volume of the natural canals were not significantly different from the printed canals [66]. However, for the validation of their model, only one 3D-printed tooth was compared with the respective natural tooth, creating six experimental pairs. So, with this methodology and with such a small number of 3D-printed teeth, it cannot be concluded that the 3D printing of the internal root anatomy is consistent when the same tooth is printed on a large scale.

Xu et al. 2021 evaluated the shaping ability of four single-file systems on 3D-printed teeth, using the PolyJet technique. Micro-CT scans were executed before and after instrumentation for each specimen [59]. One issue with this methodology is that, since the 3D-printed teeth were not compared with the natural teeth, the consistency of the printing technique cannot be assumed, and the pre- and post-instrumentation micro-CT scans continue to be needed. One objective of 3D-printed teeth is also the diminishing time and costs, which was not accomplished in this study.

The major concern is the difference in radiopacity and hardness between the resin and dentin as referred in most studies [2,23,26,55,58]. In the study of Reymus et al. 2019, students considered that the preparation of 3D-printed teeth was easier than the preparation than the natural tooth as a consequence of this difference in hardness [26]. However, it should be noted that the natural tooth was prepared upan ISO file 20 before the scan to obtain the STL file which could also have contributed to an easier preparation by the students.

A recent study by Cresswell-Boyes et al. 2022 compared 3D-printed typodont teeth with materials developed to mimic both the morphology and mechanical response of natural teeth. Their results indicate that 3D-printed typodont teeth replicating enamel and dentine can be mechanically comparable to extracted human teeth despite the material compositions differing from the materials found in human teeth [76]. Nonetheless, they used a 50 µm layer printer and only printed a typodont tooth without internal anatomy; however, these results are promising but more studies are needed in order to determine if these materials could be used in future 3D-printed teeth endodontic studies.

Another concern is, since the minimum layer thickness of 3D printers is 16 µm, the initial diameter of the root canal of the natural teeth to be printed cannot be smaller than ISO-size 15 [23], but if taken into consideration that most apical foramen diameters vary between 0.20 and 0.29 mm [45], this is perhaps not an issue. However, smaller structures such as lateral canals and narrow isthmuses probably cannot be reproduced [26,56].

The process of making 3D-printed teeth involves various steps, each of which can be a source of error [75]. For developing 3D-printed teeth, a variety of powerful freeware and open-source software solutions are available [31,66,75]; once more, the lack of standardization and the absence of protocols promote different methods of measurement and accuracy of studies making it impossible to compare the results of different studies [66].

Another question is the resolution of the scan needed for obtaining high-quality 3D-printed teeth. Kulczyk et al. 2019 compared the external surface of 3D-printed teeth obtained by different methods of 3D data acquisition, namely by CBCT, optical 3D scanning and micro-CT. They concluded that even though CBCT seems to be a sufficient method to obtain data for 3D-printed teeth, it does not equal micro-CT. They scanned a canine and a molar, and the number of triangles of the data collected by CBCT in high resolution were 65,340 and 98,624 for each tooth, respectively. With micro-CT, the numbers were 11,510,788 and 24,974,112 for each tooth, respectively. As stated before, the higher the resolution of the original data, the better the quality of the 3D model and the better the quality of the 3D-printed object. Nonetheless, 3D models created based on micro-CT data provide better accuracy than 3D printers have been able to produce to date [75]. Additionally, a very-high-resolution STL creates an excessively large file that is difficult for 3D printing software to process (Figure 2).

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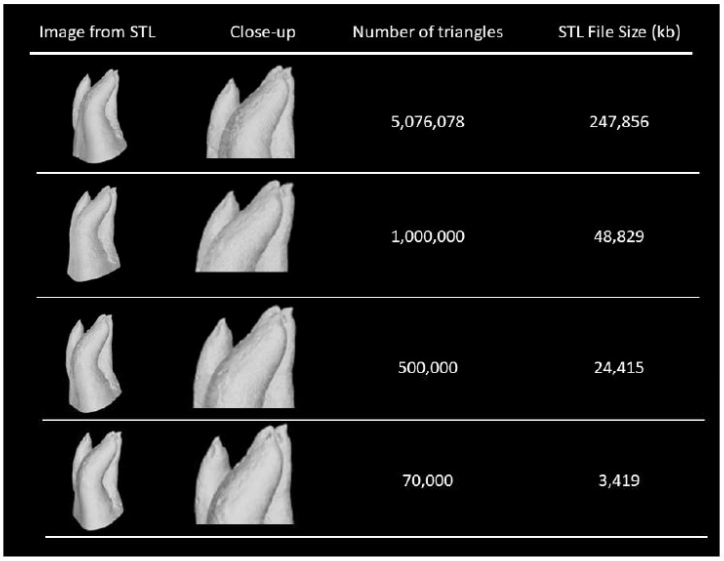

Figure 2. Differences between the number of triangles and size in kilobytes (Kb) of Standard Tessellation Language (STL) files of the same maxillary first molar.

5. Future

As previously stated, the main goal of using 3D-printed teeth is standardization. For the scientific community, this means that the possibility of creating equal protocols for ex vivo studies could allow an easy comparation of results. For the teaching community, the fact that students all have the same object of training could allow a fair evaluation and an equal progression, since every student would have the same tooth with the same degree of difficulty according to the teaching stage. We should keep in mind that for 3D-printed teeth to make any sense, it is necessary to print these teeth on a large scale, rapidly and with low associated costs.

So, to achieve the degree of standardization needed and the establishment of protocols, it is the author's opinion that the problems with 3D-printed teeth presented here should first be answered in this order, and they are summarized in Table 1.

(1) From the great plethora of existing commercial printing materials, it should be established which materials are more dentin-like. Through the fabricant datasheet, we can choose the potential materials available and compare their physical and mechanical properties and radiopacity with human dentin. This way, subsequent studies will only use the material most appropriate for each printing technique.

(2) The establishment and validation of a protocol, for each printing technique, of the total removal of the support material in 3D-printed teeth with unaltered canal anatomy could be easily achieved if the 3D-printed teeth have an access cavity already created. In sequence, establishment is the printing technique that produces consistent 3D-printed teeth with the best accuracy by comparing the internal anatomy of the natural tooth with a large number of 3D-printed teeth by using micro-CT.

(3) Investigate whether the support material influences the instrumentation technique outcomes. This could be performed by comparing teeth in which the support material was removed with teeth where the support material was not removed. If the support material does not influence the variables of interest, the support material could probably mimic the pulp tissue and could permit the printing of a 3D tooth with a closed crown and an unaltered internal root anatomy.

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(4) Evaluate the minimum and maximum number of triangles in STL files needed for maintaining the accuracy of the 3D-printed teeth. From the literature review, it is known that the higher the resolution of the original data, the better the quality of the 3D model and the better the quality of the 3D-printed object. In fact, we do not know if the large number of triangles obtained by micro-CT is necessary to achieve the necessary accuracy of the canal anatomy. Resources are perhaps being overused, since, as stated before, a very-high-resolution STL file is difficult for a 3D printing software to process. Additionally, it is necessary to establish well-defined and well-described step-by-step protocols for a reasonable number of software programs needed for the entire process of 3D printing. These protocols would eliminate a great number of confounding factors since errors can be introduced during image acquisition, image segmentation and 3D image construction.
(5) After addressing all the problems with the printing techniques, an ideal 3D printing material containing hydroxyapatite or ceramic particles that can mimic the radiopacity and/or hardness of human dentin could be developed.

Table 1. Recommendations for future research of 3D-printed teeth in endodontics.

Problems	Future
Differences in radiopacity and hardness between resin and dentin	Establish from the commercial printing material which of them are more dentin-like Develop an ideal 3D printing material containing hydroxyapatite that could mimic human dentin
Removing support materials from root canal system Validation of printing accuracy of internal anatomy	Establishment and validation of a protocol for the total removal of support material from root canal system Investigate the influence of support material in the instrumentation technique outcome
Variety of powerful freeware and open-source software solutions available	Establish well-defined and well-described step-by-step protocols for a reasonable number of software programs
Resolution of STL files and size	Evaluate what is the minimum and maximum number of triangles in STL files needed for maintaining the accuracy of 3D-printed teeth

STL—Standard Tessellation Language.

6. Conclusions

Three-dimensional-printed teeth may become the gold standard in ex vivo studies and in endodontic training, substituting natural teeth. Nonetheless, to realize this, more studies are needed to resolve the problems that 3D-printed teeth still present. Additionally, more efforts should be made to develop well-established protocols so the standardization of methods can be achieved, and then the results can be compared between studies.

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APPENDIX 4 – PERMISSION TO REPRODUCE PAPER

Title: Root Canal Preparation of a Commercial Artificial Tooth versus Natural Tooth - A MicroCT Study

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Root Canal Preparation of a Commercial Artificial Tooth versus Natural Tooth—A MicroCT Study

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Abstract: This study aimed to evaluate by microCT the preparation of the artificial teeth (ATs), TrueTooth®, versus natural teeth (NTs): (1) the time and number of pecking movements needed for preparation; (2) the root canal volume increase; (3) if the pulp-colored medium has any effect on the 3D analysis. Material and Methods: Artificial and natural maxillary molars were used. Fourteen AT distobuccal canals and fourteen NT buccal canals were used for the first and second aim and fourteen AT mesiobuccal canals for the third aim. Results: No statistically significant differences were observed regarding the time and number of pecking movements ($p > 0.05$); for the root canal volume increase, a statistically significant difference was observed ($p < 0.05$) with a higher mean value for NTs; however, in the group of ATs, there was a volume decrease in three cases. The AT mesiobuccal root canal mean volume increase was also negative. Conclusions: There are no differences between the time and number of pecking movements between NTs and ATs, so TrueTooth® can potentially be used in endodontic training. The volume increase between ATs and NTs was higher in NTs. However, some samples showed negative values, also seen in the AT mesiobuccal canal, confirming that the pulp-colored medium has an effect on the 3D analysis.

Keywords: endodontics; microCT; TrueTooth®; 3D printing; 3D analysis; endodontic training

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1. Introduction

Bacteria are the main cause of pulpal and periapical pathologies. Therefore, all endodontic procedures aim to eliminate pulp tissue, as well as as many bacteria as possible [1]. Root canal mechanical preparation is an important step to achieve this goal while simultaneously increasing the root canal volume to facilitate the decontamination of the root canal system by irrigants and medicaments [2,3]. To accomplish this, a variety of manual and rotary instruments have been proposed. The revolution in the manufacturing of root canal instruments has led to a great variety of these endodontic instruments when comparing the design, alloys, and type of movement.

Many new systems are available on the market, and clinicians require an impartial evaluation of their characteristics to help them in selecting systems to use clinically [1,2,4]. ProTaper Gold® (Dentsply Sirona) (PTG) uses control memory wire, which has a lower percentage of nickel (by weight), and because of the thermic treatments used during the manufacturing process, it does not rebound to its original shape after being flexed; this characteristic reportedly increases its flexibility [1–4].

Among the most-important basic topics under research concerning root canal preparation are: (1) the removal of dentin and the volume increase; (2) straightening and transportation; (3) unprepared root canal areas; and (4) working time [5,6].

Traditionally, for ex vivo studies and pre-clinical courses, extracted human teeth have been the standard practice [7–9]. However, natural teeth (NTs) have several drawbacks, which have been discussed in recent years: they are difficult to collect, and there are ethical considerations, potential cross-infection risks, storage drawbacks, and standardization issues [7,8,10,11]. When using NTs, the external and internal anatomy should be fairly balanced between the groups to avoid any anatomical bias [7,8,11,12], or studies might demonstrate the effect of canal anatomy rather than the variable of interest [13]. In the teaching environment also, students normally comment that the anatomical variability does not allow a valid assessment of their individual performance [14].

The most-common method used in ex vivo studies is to create pair-matched tooth samples according to their anatomical features, such as the tooth type, root length, root anatomy, degree and angle of curvature, and root canal diameter, or even with paired contralateral teeth [7,12].

Many methods are used for anatomy assessment, namely visual examination, radiographs, and micro-computed tomography (microCT). MicroCT allows a non-invasive, non-destructive, high-resolution, and 3D investigation [7]. De-Deus et al. (2020) compared the randomized method, radiographic method, and microCT method and concluded that microCT was able to provide better control of the confounding effects of anatomical variance. Nonetheless, this study also demonstrated the difficulty, time consumption, and high costs of this method [12]. Additionally, it should be noted that other tooth parameters should be taken into count, which cannot be sufficiently standardized by these methods, such as the age and ethnicity of the donor and environmental factors. These factors have an impact on the mechanical properties of the dentin, so a total standardization is, with the actual methods, almost impossible to achieve [15].

Three-dimensionally printed teeth that reproduce the features of natural teeth may overcome all these limitations [10,11,16] and are also suitable for practicing access opening, canal instrumentation, radiographic length control, and canal filling [8,16]. They are realistic and standardized, so the same level of difficulty is guaranteed for every single student, and consequently, they will be scored fairly [8–10,16]. Additionally, in ex vivo studies, this morphological standardization has a major impact on the results' credibility, since anatomical bias would not exist [12,13].

In the study of Gancedo-Caravia et al. (2020), it was concluded that replicas obtained from microCT scans better reproduced the internal anatomy, and in particular, TrueTooth® was the best replica for access cavity preparation training and root canal negotiation [5]. TrueTooth® (DELabs) is a 3D-printed replica of human teeth, designed for teaching access, negotiation, shaping, irrigation, and obturation for root canal iterative training [17], that is radio-opaque with a pulp-colored medium inside each canal space, which can be dissolved with sodium hypochlorite (NaOCl) [18]. TrueTooth® replica #15-001 is a maxillary molar with a distobuccal canal with a severe curvature and a large mesiobuccal canal, ribbon-shaped, with a moderate curvature [17]; these features are adequate for the purposes of the present study.

Nonetheless, it has been reported that, in 3D-printed teeth, it is difficult to remove the support material from parts with features having fine details [7,8]. In this way, canals could be filled partially or totally with the support material, and the effect of this material on microCT 3D analysis should be considered [15].

To the authors' knowledge, there are no studies that (1) compare the time and pecking movements needed for the preparation of TrueTooth® with NTs, (2) investigate if TrueTooth® is adequate for the evaluation of the canal preparation using microCT, and/or (3) analyze the effect of the support material on the variables of interest.

Hence, the aim of this study is to evaluate the preparation of TrueTooth® with the PTG system and compare this to NTs in the following aspects: (1) the time and number

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of pecking movements needed for the root canal preparation; (2) the root canal volume increase; and (3) if the pulp-colored medium has any effect on the 3D analysis by microCT in order to assess if TrueTooth® can be used in endodontic training and/or microCT studies. The null hypothesis is that the time, pecking movement, and root canal volume increase are equal between TrueTooth® and NTs and that the TrueTooth® pulp-colored medium has no effect on the variables of interest.

2. Materials and Methods

2.1. Specimen Selection

Based on previous studies [19–21], a sample size calculation was performed using the G*Power 3.1.9.7 software for windows (Heinrich Heine, Universität Düsseldorf, Germany), and the *t*-test for 2 independent groups was used with an α-type error of 0.05 and a β power of 0.80 (a large effect size equal to 1 was considered for sample size calculation for all variables), resulting in a required size of 14 samples per group.

2.2. Natural Specimen Selection

This research work, with ethics committee approval (IAO-23-0104), used an initial pool of 65 maxillary permanent molars, extracted for reasons unrelated to this study, collected and stored in distilled water until use. Radiographs were taken in the mesiodistal and buccolingual directions to ensure that the inclusion criteria were met. The inclusion criteria were teeth with fully formed apices, the absence of root fractures, no signs of external and internal resorption or decayed tissue in the region of interest, the absence of previous endodontic treatment, the absence of calcifications, teeth with separated canals and apical foramens, and a degree of curvature between 20° and 50°. The degree of curvature was measured according to Schneider's method [22]. It was determined drawing two lines: the first was parallel to the long axis of the canal, and the second line was drawn from the apical foramen to intersect with the first at the point where the canal began to leave the long axis of the tooth. Based on these criteria, and to economize the resources, the buccal canals of 7 teeth were selected. All the preparations were performed by a single operator with 15 years of clinical experience in the field of endodontics and previous experience in PTG system use. The teeth were mounted in place using the ProTrain system® (Simit Dental, Mantova, Italy).

The endodontic cavities were prepared using a round diamond bur #4 and an Endo-Z™ bur (Dentsply-Maillefer, Ballaigues, Switzerland) driven by a high-speed handpiece under water cooling. The buccal canals were explored by advancing passively into the canal a K-file #10 (Dentsply Sirona) until the tip of the file was just visible through the apical foramen. The working length (WL) was determined by reducing 1 mm from that value.

2.3. Artificial Specimen Selection

For the AT group, fourteen TrueTooth® replicas (#15-001) were used in the study. The 14 distobuccal canals were used for comparison with the 14 natural buccal canals, and the 14 mesiobuccal canals were used to access the effect of the pulp-colored medium. ATs were prepared equally to NTs.

2.4. Initial MicroCT Scanning

The specimens were then scanned using a microCT device (Skyscan 1174; Bruker, microCT) using microCT imaging at 50 KV and 800 mA energy, and a 0.25 mm-thick aluminum filter was used with rotational steps of 1° increments for a total rotation of 180° with a 16.65 µm image pixel size.

The degree of homogeneity between the groups, regarding the initial volume, WL, and degree of curvature was assessed and confirmed statistically ($p > 0.05$), indicating adequate paring of NT buccal canals and AT distobuccal canals.

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2.5. Root Canal Preparation

An electric motor (X-Smart® Plus, Dentsply Sirona) was used to operate the files with in-and-out pecking motion (2–3 mm amplitude), in a continuous clockwise rotation, according to the manufacturer's recommendations. The number of pecking movements for each file needed to reach the WL and the time that the file was rotating inside the canal until reaching the WL were recorded, using a start-stopwatch. If the file did not reach the WL with 3 pecking movements, the file was removed from the canal to clean the debris from the flutes. During this period, the start-stopwatch was stopped. A glide path was created by using a ProGlider® instrument (PG) (Dentsply Sirona) until the WL was reached. All files from the PTG system (Dentsply Sirona) were used up to the WL in the sequence Sx, S1, S2, F1, and F2 (tip size 25, 0.08 taper). Patency was checked after use of each instrument with a K-file #10 (Dentsply Sirona). For all groups, the instruments were used to prepare only two canals, and after that, they were discharged. Root canal irrigation was performed between each file with 5.25% NaOCl (Cerkamed) using an IrriFlex® 30G needle (PD Dental) inserted in the canal as apically as possible. A total of 25 mL was used to irrigate each canal. After drying the canals with absorbent paper points F2 PTG, a new microCT was performed according to the same scanning and reconstruction parameters as those established initially.

2.6. MicroCT Analysis

Images were reconstructed using the NRecon v 1.7.46 software (Bruker microCT) and superimposed with geometric alignment using the DataViewer v 1.5.6.2 software (Bruker microCT). The CTAnv v 1.20.3.0 software (Bruker microCT) was applied to calculate the quantitative parameters. The region of interest was set for each sample from the furcation region to the apex of the root.

2.7. Total Time of Preparation

The times that each file took to reach the WL (PG and all the files from the PTG system) were added to obtain the total time of preparation needed for each canal.

2.8. Total Number of Pecking Movements

The numbers of pecking movements needed for each file (PG and all the files from the PTG system) to reach the WL were added to obtain the total number of pecking movements needed for the preparation of each canal.

2.9. Root Canal Volume Increase

The initial volume (IV) and final volume (FV) were obtained, the IV being the preoperative volume of the root canals and the FV being the volume of the root canals after the preparation. Based on these values, the root canal volume increase was calculated using the following formula: FV − IV.

2.10. Statistical Analysis

The data collected were processed using the IBM SPSS Statistics vs. 26.0 software, considering a significance level of 0.05 in all situations of statistical inference. Statistical analysis was performed using the Mann–Whitney U-test for 2 independent groups.

3. Results

Table 1 shows the mean, minimum, and maximum values of the total time of the preparation and the total number of pecking movements needed for the NT buccal canals' and the AT distobuccal canals' preparation. No statistically significant differences were observed between the NTs and ATs regarding the time of preparation and number of pecking movements ($p > 0.05$).

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Table 1. Mean, minimum, and maximum values of the time of preparation and number of pecking movements for natural tooth buccal canals and artificial tooth distobuccal canals.

Parameter		Natural Teeth	Artificial Teeth
Total time of preparation (s)	Mean Min–Max	64.3 (52–87)	63.5 (51–71)
Total number of pecking movements	Mean Min–Max	33.50 (26–38)	25.86 (12–44)

s, seconds; Min, minimum; Max, maximum.

Table 2 shows the mean, minimum, and maximum values of the initial root volume, final root volume, and root canal volume increase of NT buccal canals and AT distobuccal canals. A statistically significant difference was observed between NTs and ATs ($p < 0.05$) with a higher mean value for NTs; however, it should be noted that, in the group of ATs, there were negative values for this parameter.

Table 2. Mean, minimum, and maximum values of the initial volume, final volume, and volume increase in natural tooth buccal canals and artificial tooth distobuccal canals.

Parameter		Natural Teeth	Artificial Teeth
Initial root canal volume (mm^3)	Mean Min–Max	2.68 (0.52–7.44)	2.70 (2.56–2.82)
Final root canal volume (mm^3)	Mean Min–Max	4.07 (1.42–9.91)	3.40 (2.08–4.15)
Root canal volume increase (mm^3)	Mean Min–Max	1.39 (0.34–2.77)	0.71 (-0.74–1.51)

mm^3, cubic millimeters; Min, minimum; Max, maximum.

Table 3 shows the mean, minimum, and maximum values of the initial root volume, final root volume, and root canal volume increase of the AT mesiobuccal canals to evaluate the effect of the pulp-colored medium. The 3D analysis of the AT mesiobuccal root canal is illustrated in Figure 1 (before tooth preparation) and Figure 2 (after tooth preparation). For the effects of the comparison, Figure 3 (before tooth preparation) and Figure 4 (after tooth preparation) illustrate a mesiobuccal root canal of an NT.

Table 3. Mean, minimum, and maximum values of the initial volume, final volume, and volume increase of artificial tooth mesiobuccal canals.

Parameter		Artificial Tooth Mesiobuccal Root Canal
Initial root canal volume (mm^3)	Mean Min–Max	7.45 (7.00–8.05)
Final root canal volume (mm^3)	Mean Min–Max	6.14 (5.30–7.86)
Root canal volume increase (mm^3)	Mean Min–Max	−1.32 (−2.14–0.86)

mm^3, cubic millimeters; Min, minimum; Max, maximum.

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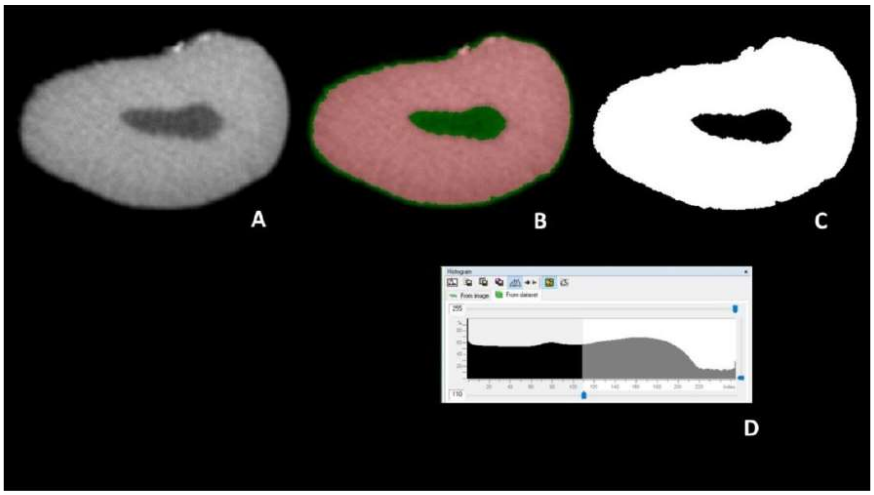

Figure 1. AT mesiobuccal root before instrumentation: (**A**) raw image from microCT dataset; (**B**) image with threshold applied; (**C**) binarized black and white image; (**D**) histogram with selected threshold values.

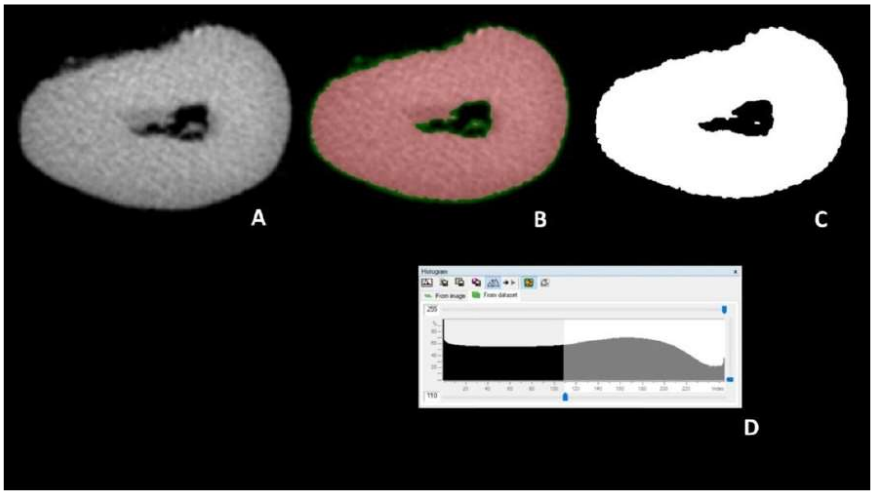

Figure 2. AT mesiobuccal root after instrumentation: (**A**) raw image from microCT dataset; (**B**) image with threshold applied; (**C**) binarized black and white image; (**D**) histogram with selected threshold values.

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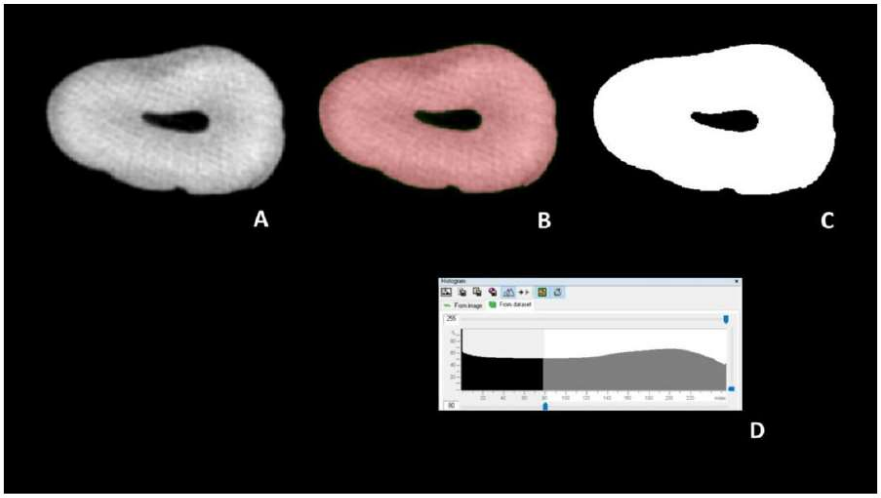

Figure 3. NT mesiobuccal root before instrumentation: (**A**) raw image from microCT dataset; (**B**) image with threshold applied; (**C**) binarized black and white image; (**D**) histogram with selected threshold values.

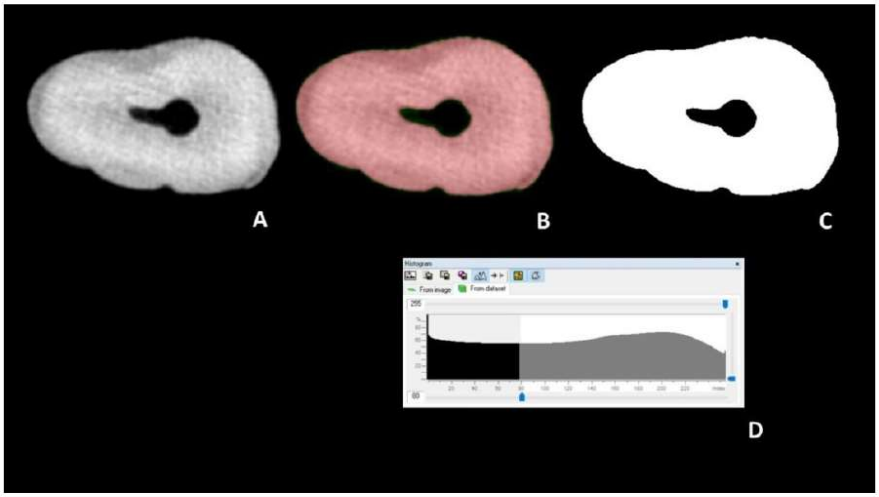

Figure 4. NT mesiobuccal root after instrumentation: (**A**) raw image from microCT dataset; (**B**) image with threshold applied; (**C**) binarized black and white image; (**D**) histogram with selected threshold values.

4. Discussion

The first aim of this study was to evaluate the time and number of pecking movements needed to prepare NT canals versus AT canals. The mean time of preparation for ATs was one second less, and they needed fewer pecking movements for canal preparation. These findings indicate that, although being faster and needing fewer pecking movements to prepare the AT canals, perhaps due to the difference in the hardness between resin

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and human dentine [7,10,11], there were no statistically significant differences between them. Therefore, we accepted the null hypothesis. This result is in accordance with the results observed in the study of Luz et al. (2015), which compared the difference of the time of preparation needed by students and specialists, using hand-files, between NTs and ATs. They concluded that the time needed to prepare ATs was lower than NTs, in both groups [23].

In this study, the mean value of the preparation time for NTs was 64.3 s with the PTG system, which is less compared to the study of Arslan et al. (2017), which showed a mean value of 78 s. Nonetheless, the authors did not make any reference to whether the glide path was performed manually or mechanically or even if it was performed, and this could explain the difference between the two values [24]. The literature shows that a mechanical glide path is faster than a manual glide path [25,26]; furthermore, if a glide path is not performed, the root preparation time is longer [27].

In the study of Bitter et al. (2016), 43 students were divided in two groups; one group only trained on ATs (TrueTooth®), the test group, and the other on NTs, the control group. The students had to attend a training period, and after this period, both groups prepared an NT and an AT and were evaluated. It was concluded that students solely trained on ATs did not perform significantly different on NTs than those trained on NTs; nonetheless, in general, the performance of students tended to be better on ATs than NTs [9]. Furthermore, in the study of Gancedo-Caravia et al. (2020), students were asked to perform endodontic treatments on different commercial ATs, and then, their outcome was evaluated by three calibrated experienced endodontic educators. The ATs were compared from three perspectives: students' achievements, students' perceptions, and educators' perceptions. The educators considered that replicas obtained from microCT scans better reproduced the internal anatomy, and specifically, TrueTooth® was the best replica for the training of access cavity preparation and negotiation. On the other hand, students rated and performed on TrueTooth® less well due to the greater level of difficulty compared to ATs [5]. The results of these two studies, and the results of the present study, regarding preparation time and the number of pecking movements needed for root canal preparation support that TrueTooth® can potentially be used for endodontic training. However, future studies are needed using different preparation systems, with different geometries and movements of preparation.

The second aim of this study was to evaluate if the root canal volume increase was similar between ATs and NTs. In fact, there was a significant statistical difference between ATs and NTs, the root canal volume increase being higher in NTs. However, as we stated before, after the canal preparation of ATs, some samples showed negative values; this would mean a loss of volume after preparation, and this situation seems absurd. With these results, the null hypothesis cannot be accepted nor rejected, and these results must be observed in conjunction with the results of the 3D analysis of the AT mesiobuccal canal.

Nonetheless, in our study, the mean value for the root canal volume increase in the NT group was 1.39 mm^3. In the literature, the root canal volume increase using the PTG system ranges from 1.33 to 2.77 mm^3 [1,2,28,29]. Nonetheless, it has been shown that variations in canal geometry before preparation may have a greater effect on the observed changes than the preparation techniques themselves [30], and this can explain the differences between the results of the studies.

In a literature review, Reis et al. (2022) reported the problems that 3D-printed teeth still present and the need to answer these problems before these teeth can be used routinely in ex vivo studies. One of the problems presented is the absence of studies that describe a protocol for support material removal and/or the effect of this material on the variables of interest [15]; in this way, our third aim was to assess if the pulp-colored medium had any effect on the 3D analysis by microCT, and it was observed that the mean value of the root canal volume increase was negative, confirming the results of the comparison of the volume increase between ATs and NTs; in this way, the pulp-colored medium had an effect on the 3D analysis. Therefore, we cannot accept the null hypothesis. As illustrated in Figures 1 and 2, the density of the pulp-colored medium, after preparation, was higher than

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before canal preparation, and its density became like the density of the resin that comprises the root. Therefore, after canal preparation, the 3D analysis software cannot separate and identify these as two different materials. Nonetheless, prior to the preparation, this was possible. This situation did not occur in NTs, as seen in Figures 3 and 4, since the pulp tissue existing in the canals before the preparation is not a confounding material for the 3D analysis software.

To the author's knowledge, there are no studies that have evaluated this feature with microCT, so we cannot compare our results to others. We hypothesized that the density changes may be due to the friction heat of the files rotating inside the canal and that the pulp-colored medium is condensed against the root canal walls in the areas that the files do not touch. This friction heat has been described and used in non-surgical endodontic retreatments [31]. When the results between the AT mesiobuccal and distobuccal canals were compared, the negative results were greater in the mesiobuccal canal; as mentioned before, this is a large ribbon-shaped canal, and it has been reported that oval and long oval root canals are difficult to prepare, because there is a tendency for the file to remain in the center of the canal and not reach all the dentinal walls, which does not allow an adequate preparation [32,33]. This could explain these differences. For a comparison with our results, future studies using TrueTooth® are needed, using, for example, different irrigation protocols, systems, and movements of preparation and also different TrueTooth® replicas with other canal anatomies.

Regarding non-commercial 3D-printed teeth, it was stated that there are no studies that have demonstrated that the internal root canal anatomy is free of support material, and most of the studies did not explain the protocol used for removing it from inside the root canals or even if this was performed [34–39]. In this way, canals could be filled partially or totally with the support material [15]; our findings for this situation pose questions about the results of ex vivo studies that use 3D-printed teeth.

In this way, based on our findings, it is our opinion that ATs, commercial or non-commercial, can be routinely used in future microCT studies without raising these questions about the results' validity, and a prior evaluation of the support material effects should be performed for each type of AT. Since the support material is different in different printing techniques/materials, future studies with 3D-printed teeth should always present a well-described support-material-removal protocol and demonstrate that the ATs used in the respective study are support-material-free or at least demonstrate that the used AT support material has no effect on the variables of interest.

5. Conclusions

There were no statistically significant differences between the time and number of pecking movements between NTs and ATs, so it can be concluded that TrueTooth® is an adequate replica that can potentially be used in endodontic training. There was a significant statistical difference between ATs and NTs, the root canal volume increase being higher in NTs. However, some samples showed negative values, and in the 3D analysis of the AT mesiobuccal canal, the mean value of the root canal volume increase was negative, confirming that the pulp-colored medium had an effect on the 3D analysis.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee (Comité de Ética) of Instituto Asturiano de Odontologia (IAO-23-0104).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions (undergoing PhD thesis).

Conflicts of Interest: The authors declare no conflict of interest.

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Three-Dimensional Printed Teeth in Endodontics: A New Protocol for Microcomputed Tomography Studies

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Abstract: This study aimed to describe a support material removal protocol (SMRP) from inside the root canals of three-dimensional printed teeth (3DPT) obtained by the microcomputed tomography (microCT) of a natural tooth (NT), evaluate its effectiveness by comparing the 3DPT to NT in terms of internal anatomy and behaviour toward endodontic preparation, and evaluate if 3DPT are adequate to assess the differences between two preparation systems. After the SMRP, twenty 3DPT printed by PolyJet™ were microCT scanned before preparation and thereafter randomly assigned into two groups (n = 10). One group and NT were prepared using ProTaper Gold® (PTG), and the other group with Endogal® (ENDG). MicroCT scans were carried out after preparation, and the volume increase, volume of dentin removed, centroids, transportation, and unprepared areas were compared. For the parameters evaluated, no significant differences were found between the 3DPT and NT before and after preparation ($p > 0.05$), and no significant differences were found between the 3DPT PTG group and the 3DPT ENDG group ($p > 0.05$). It can be concluded that the SMRP described is effective in removing the support material SUP706B™. PolyJet™ is adequate for printing 3DPT. Furthermore, 3DPT printed with high-temperature RGD525™ have similar behaviour during endodontic preparation with PTG as the NT, and 3DPT can be used to compare two preparation systems.

Keywords: endodontics; three-dimensional printed teeth; PolyJet; support material; microcomputed tomography

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1. Introduction

The American Association of Endodontists defines root canal preparation as "Procedures involved in cleaning and shaping the canal system prior to obturation", distinguishing between "biomechanical preparation" as the "use of rotary/reciprocating and/or hand instruments to expose, clean, enlarge and shape the pulp canal space, usually in conjunction with irrigants" and "chemomechanical preparation" as the "use of chemicals for irrigation of the root canal, demineralization of dentin, dissolution of pulp tissue and neutralization of bacterial products and toxins; used in conjunction with biomechanical preparation" [1].

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Over the last few decades, root canal preparation protocols have changed, and many new nickel–titanium systems have become available; nonetheless, clinicians require knowledge of shaping properties and a performance evaluation of these systems to select them according to clinical cases [2]. Root canal preparation ex vivo studies provide useful and valuable data to improve the biological outcome of preparation and therefore have to be continued in the future [3].

Extracted human natural teeth (NT) are considered the gold standard for ex vivo studies; however, they present several disadvantages, the main one being very difficult standardization [4], not only as a consequence of the root canal system anatomy but also the donor's age, which has an influence on the dentin properties [5,6]. Three-dimensional printed teeth (3DPT) obtained through the microcomputed tomography (microCT) of real NT have been used as an alternative for NT in both research and teaching and offer a good opportunity to create balanced experimental groups [3,7–14]. The major concern expressed about 3DPT is the difference in radiopacity and hardness between resin and human dentin [3,7,9,10,12]. Nonetheless, protocols for the standardization of studies using 3DPT still have to be developed [3].

PolyJet® (Stratasys Ltd., Eden Prairie, MN, USA) printing is based on layer-by-layer technology. The process consists of the nozzles of the printer moving along the XY plane and spraying liquid photosensitive resin on the printer bed, and a UV lamp cures the resin. After the first layer is finished, the printer bed will drop by a layer thickness in the Z plane, and the deposition of another layer is repeated. This is a potentially attractive option for low-volume manufacturing in research environments. Where hollow parts or overhangs exist, the nozzles spray a layer of removable support material [15–17]. Support material removal methods include manual breaking, dissolution under water pressure or with sodium hydroxide (NaOH), or melting [15,18,19]. However, it has been reported that there is difficulty in removing the support material inside 3DPT root canals [3,7,8,17,20], so canals could be filled partially or totally with support material, which may have an effect on microCT analyses before and/or after preparation [21].

In order for 3DPT to be used in ex vivo preparation studies, their internal anatomy should be similar to the original NT, they should be support-material-free, their behaviour during preparation should be identical to that of NT, and they should be capable of assessing the differences between two preparation systems. To the authors' knowledge, there are no studies that describe a protocol for removing the support material from inside the root canals of 3DPT printed by PolyJet™. In addition, the available literature regarding the printing accuracy of internal anatomy is still scarce.

Therefore, the first aim of this study was to evaluate the effectiveness of a support material removal protocol (SMRP) for inside root canals by comparing the internal anatomy of a NT with 3DPT. The null hypothesis was that there was no difference between the internal anatomy of NT and 3DPT. The second aim of this study was to compare the behaviour under preparation with ProTaper Gold® (Dentsply-Sirona, Fair Lawn, NJ, USA) (PTG) between NT and 3DPT. The null hypothesis was that there was no difference in preparation behaviour between NT and 3DPT. The third aim of this study was to compare the behaviour of 3DPT during preparation with two different preparation systems, namely PTG and Endogal® (Endogal, Galician Endodontics Company, Lugo, Spain) (ENDG). The null hypothesis was that no difference existed between PTG and ENDG.

2. Materials and Methods

The study protocol was approved by the ethics committee of Fernando Pessoa University (FCS/PI 429/23). Based on data from a previous study [22] in which shaping ability was assessed, a sample size calculation was performed using G*Power 3.1.9.7 software for Windows (Heinrich Heine, Universität Düsseldorf, Düsseldorf, Germany) with an α type error of 0.05 and a β power of 0.95 for an effect size of 1.79 input into the *t* test family, resulting in a required sample size of 16 samples (8 per group) to observe significant

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differences between groups. Ten samples were used per group to compensate for possible sample loss during experimental procedures.

2.1. Natural Specimen Selection

An initial pool of 45 maxillary permanent molars, extracted for reasons unrelated to this study, was used. The teeth were collected and stored in distilled water until use. Radiographs were taken in mesiodistal and buccolingual directions to ensure that the inclusion criteria were met. The inclusion criteria were teeth with fully formed apices, the absence of root fractures, no signs of external and internal resorption or decayed tissue in the region of interest, the absence of previous endodontic treatment, and a degree of curvature between 20° and 40°. The degree of curvature was measured according to Schneider's method [23]. From the initial sample, 14 teeth were selected. The endodontic cavities were prepared using a round diamond bur #4 and an Endo-Z™ bur (Dentsply Sirona, Fair Lawn, NJ, USA) driven by a high-speed handpiece under water cooling. The canals were explored with a K-file #10 (Dentsply Sirona) until the tip of the file was just visible through the apical foramen to ensure the existence of canal patency. The tooth crowns were sectioned at 4 mm from the cementoenamel junction to create a platform for easing future references. The specimens were then scanned using a microCT device (Skyscan 1174; Bruker, Kontich, Belgium) at 50 KV and 800 mA energy; a 0.25 mm thick aluminium filter was used, with rotational steps of 1.0 increments for a total rotation of 180°, a 16.65 µm image pixel size, and an exposure time of 12,000 ms. Images were reconstructed using NRecon version 1.7.46 software (Bruker, Kontich, Belgium, in which algorithms were introduced for the correction of ring artefacts (3), smoothing (3), and beam hardening (40%). CTAn version 1.20.3.0 software (Bruker, Kontich, Belgium) was applied to produce one STL file of each tooth and another of the respective canal's anatomy. The STL files were exported to a free software platform (MeshLab version 2021.10) for qualitative canal configuration evaluation and the election of the NT to replicate. The tooth chosen was a second left maxillary molar that presented 3 fully separated roots, each one with a single independent canal, without any lateral canals. The mesiobuccal root presented an oval canal with a degree of curvature of 32°; the distobuccal root presented a round small diameter canal with a degree of curvature of 30°; and the palatal root presented a round large diameter canal with a degree of curvature of 26° (Figure 1).

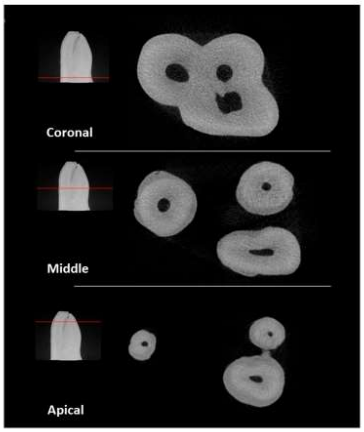

Figure 1. Cross-sectional views of microCT scan of the natural tooth.

The STL file of the tooth was simplified and prepared for 3D printing using the "Simplification: Quadric Edge Collapse Decimation" filter [14] because a high-resolution STL

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file is an excessively large file that 3D printing softwares has difficulties processing [4]. The original STL file presented 3,298,498 vertices and 6,598,466 faces with a 314 Mbytes size, and the simplified STL presented 798,881 vertices and 1,599,232 faces with a 76.2 Mbytes size. The STL file was printed by PolyJet™ using a Stratasys Object30 Prime™ printer (Stratasys Ltd., Eden Prairie, MN, USA) in high-quality mode with a layer thickness of 16 µm. High-temperature RGD525™ (Stratasys Ltd.) and the support material SUP706B™ (Stratasys Ltd.) were the materials chosen for printing. The tooth was displayed horizontally with the mesial surface in contact with the printer bed, with its long axis parallel to the X axis of the printer head and printer bed (Figure 2).

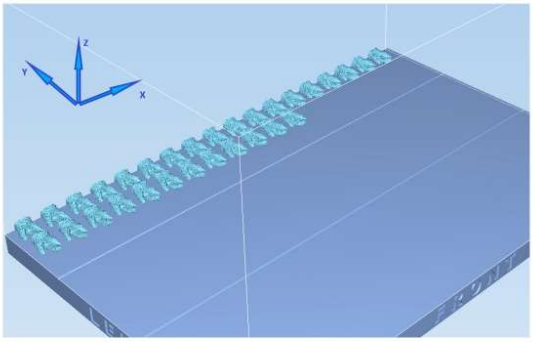

Figure 2. Teeth orientation on the printer bed.

After printing, the support material involving the teeth and inside the access cavity was removed manually. The support material removal protocol (SMRP), summarized in a flowchart (Figure 3), involved using a K-file #15 (Dentsply Sirona), a 30G polypropylene body needle Irriflex® (Produits dentaires SA, Vevey, Switzerland), and an Endoactivator® (Dentsply Sirona) with a small Endoactivator® Tip (15/0.02) (Dentsply Sirona). Irrigants were used, namely a 5% solution of NaOH, 5% Derquim® LM 01 alkalin detergent (ITW Reagents, S.R.L., Castellar del Vallès, Spain), distilled water, and 70% alcohol. The SMRP steps were (1) access cavity cleaning with 5 mL of 5% NaOH, and for each canal, (2) advance passively into the canal the K-file #15 until the tip of the file is just visible through the apical foramen to ensure the patency of the canal, followed by irrigation with 5 mL of 5% NaOH at high pressure. This step was repeated 5 times to achieve irrigant extrusion at the end of this step. Then, it was followed by (3) irrigation with 35 mL of 5% NaOH; during this step, if irrigant extrusion was lost, meaning losing the patency, a K-file #15 was used as before; (4) irrigant sonic activation with an Endoactivator® (Dentsply Sirona) at a high frequency for 30 s with up-and-down movements, with an amplitude of 4 mm; (5) irrigation with 5 mL of 5% NaOH; (6) irrigant sonic activation as described before; (7) irrigation with 5 mL of 5% Derquim® LM 01 alkalin detergent; (8) irrigant sonic activation as described before; (9) irrigation with 5 mL of distilled water; (10) irrigant sonic activation as described before; (11) irrigation with 5 mL of distilled water; and (12) irrigation with 2 mL of 70% alcohol.

In this way, a 20-3DPT sample (n = 20) randomly assigned (www.random.org, accessed on 10 January 2023) to each of the two experimental groups (n = 10), PTG and ENDG (Figure 4), was obtained.

In sequence, the 3DPT were scanned using a microCT device (Skyscan 1174; Bruker) at 50 KV; 800 mA of energy; and rotational steps of 1.0 increments for a total rotation of 180° with a 16.65 µm image pixel size and an exposure time of 12,000 ms. The images were reconstructed using NRecon v 1.7.46 software, in which algorithms were introduced for the correction of ring artefacts (5), smoothing (3), and beam hardening (50%).

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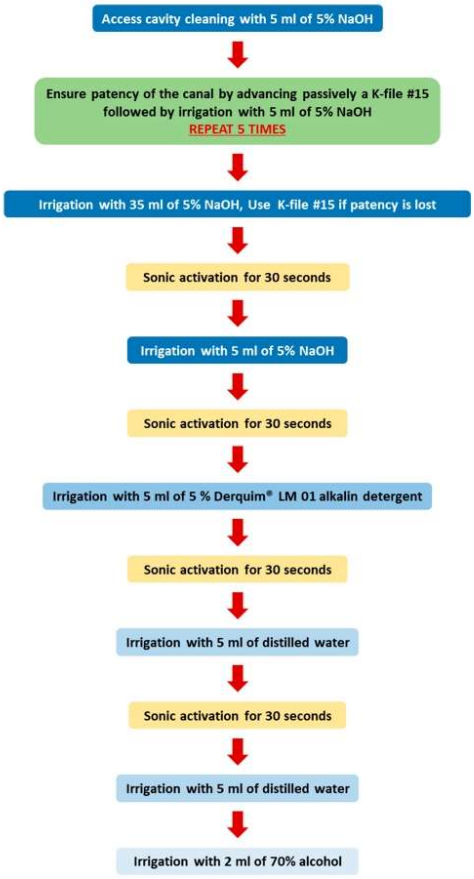

Figure 3. Flowchart of support material removal protocol.

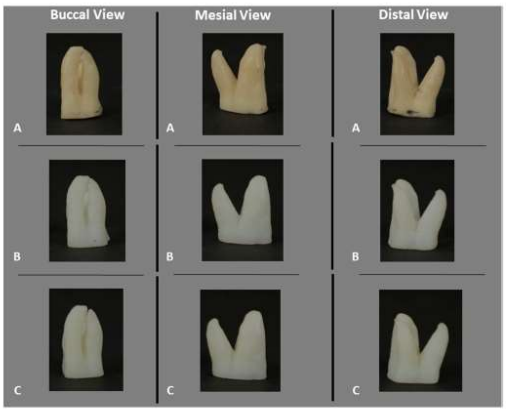

Figure 4. Natural teeth (**A**) and 3D-printed teeth (**B**,**C**) from different views.

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2.2. Root Canal Preparation

All the preparations were performed by a single operator with 22 years of clinical experience in the field of endodontics and previous experience in using PTG and ENDG systems clinically. The teeth were mounted in place using the ProTrain system® (Simit Dental Srl, Mantua, Italy). The working length (WL) was determined by taking 1 mm from the value obtained during the SMRP. The WL was 16 mm for the buccal canals and 15 mm for the palatal canal. An electric motor X-Smart® Plus (Dentsply Sirona) was used to operate the files with in-and-out pecking motion (2–3 mm amplitude) in a continuous clockwise rotation according to the manufacturer's recommendations.

2.3. Preparation of NT and Group I with PTG

A glide path was created by using a ProGlider® instrument (Dentsply Sirona) (a size tip of 16 and progressive taper from 0.02 to 0.08) until the WL was reached. All files from the PTG system, which have a convex triangular cross section and progressive taper [24], were used up to the WL except the SX file, which was used only for coronal interference removal, in the sequence SX (19/0.04), S1 (18/0.02), S2 (20/0.04), F1 (20/0.07), and F2 (25/0.08). Patency was checked after the use of each instrument with K-file #10. The instruments were used for one tooth preparation; after that, they were discharged. Root canal irrigation was performed between each file with 5 mL of 5.25% NaOCl (Cerkamed, Stalowa Wola, Poland) for NT and 5 mL of distilled water for 3DPT using an Irriflex® (Produits dentaires SA, Vevey, Switzerland) needle and activated with an Endoactivator® at a high frequency with a small tip for 30 s. After preparation, the canals were irrigated two times with 5 mL of irrigant and activated with the Endoactivator® (Dentsply Sirona), so the total volume of irrigation for each canal was 40 mL. In the end, the canals were dried with paper points (Dentsply Sirona).

2.4. Preparation of Group II with ENDG

A glide path was created by using the A (15/0.03) instrument until the WL was reached. All files from the ENDG system, which is a new system with a parallelogram cross section and instruments of constant 4% and 6% taper [25], were used up to the WL, except the X file, which was used only for coronal interference removal, in the sequence X (25/0.09), B (20/0.04), C (25/0.04), and D (25/0.06). Patency was checked after the use of each instrument with K-file #10. The instruments were used for one tooth preparation; after that, they were discharged. Root canal irrigation was performed between each file with 5 ml of distilled water using an Irriflex® needle and activated with the Endoactivator® at a high frequency with a small tip for 30 s. After preparation, the canals were irrigated three times with 5 mL of irrigant and activated with Endoactivator®, so the total volume of irrigation for each canal was 40 mL. In the end, the canals were dried with paper points.

A new microCT scan was performed on NT and both 3DPT groups according to the same scanning and reconstruction parameters as those established initially, respectively.

2.5. MicroCT Evaluation

A microCT evaluation was conducted by one of the authors, blinded to the groups. Images before and after preparation were superimposed with the 3D registration application of the DataViewer v 1.5.6.2 software (Bruker microCT), and the data obtained were processed using CTAnv v 1.20.3.0 software (Bruker microCT). The region of interest was set from the furcation region to the apex of the root.

The volume of dentine removed and centroids were quantified by subtracting the values before preparation from the values after preparation [3]. In accordance with the orientation in which the samples were evaluated, a positive value for the alteration of centroid X meant an alteration in the buccal direction, centroid Y in the mesial direction, and centroid Z in the apical direction [26]. The transportation was investigated by calculating the vectorial translocation of all sections' X, Y, and Z coordinate values using the following formula, where "a" is after and "b" is before preparation: $\sqrt{(Xa - Xb)^2 + (Ya - Yb)^2 + (Za - Zb)^2}$ [27].

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The percentage of unprepared area was calculated by the number of static voxels compared with the total number of voxels present on the root canal surface [26]. The tooth volume expansion evaluated for the 3DPT corresponds to the ratio of the 3DPT tooth volume compared to the NT volume [13].

2.6. Statistical Analysis

The collected data were processed using IBM SPSS Statistics version 26.0 software. The Shapiro–Wilk test was applied to verify data normality. Accordingly, for normal and non-normal distributions, a one-sample *t*-test or one-sample Wilcoxon signed-rank test and an independent-sample *t*-test or Mann–Whitney U test were applied. The significance level was 5% for all statistical tests ($p < 0.05$).

3. Results

The measurements of canal volume, centroid X, Y, and Z before preparation, and the percentage of tooth volume expansion for the 3DPT are shown in Table 1. There were no statistically significant differences between the NT and the 3DPT for all the variables ($p > 0.05$) (Figures 5 and 6).

Table 1. Microcomputed tomographic analysis before preparation of natural teeth and 3D-printed teeth.

Data	Natural Tooth	3D-Printed Teeth	
Canal Volume (mm^3)	12.08	Mean ± SD Median	11.99 ± 0.55 12.07
Centroid X (mm)	10.92	Mean ± SD Median	10.92 ± 0.09 10.93
Centroid Y (mm)	10.37	Mean ± SD Median	10.35 ± 0.07 10.35
Centroid Z (mm)	8.03	Mean ± SD Median	8.02 ± 0.09 8.04
Tooth Volume Expansion (%)		Mean ± SD Median	0.73 ± 0.77 0.68

A comparison between the NT and 3DPT before and after preparation with the PTG system is presented in Table 2. There were no statistically significant differences between the NT and the 3DPT for all the variables before preparation ($p > 0.05$). The 3DPT showed a similar behaviour to the NT after preparation since there were no statistically significant differences ($p > 0.05$) related to the measurements of canal volume, centroid X, Y, and Z after preparation, the volume of dentin removed, the percentage of volume increase, centroid X, Y, and Z alteration, transportation, and the percentage of unprepared areas (Figures 6 and 7).

The overall results of the measurements before preparation, as well as the results of the shaping performance of the PTG and ENDG instrument systems on the 3DPT, are presented in Table 3. There were no statistically significant differences between the two groups for all the variables before and after preparation ($p > 0.05$). (Figures 6 and 7).

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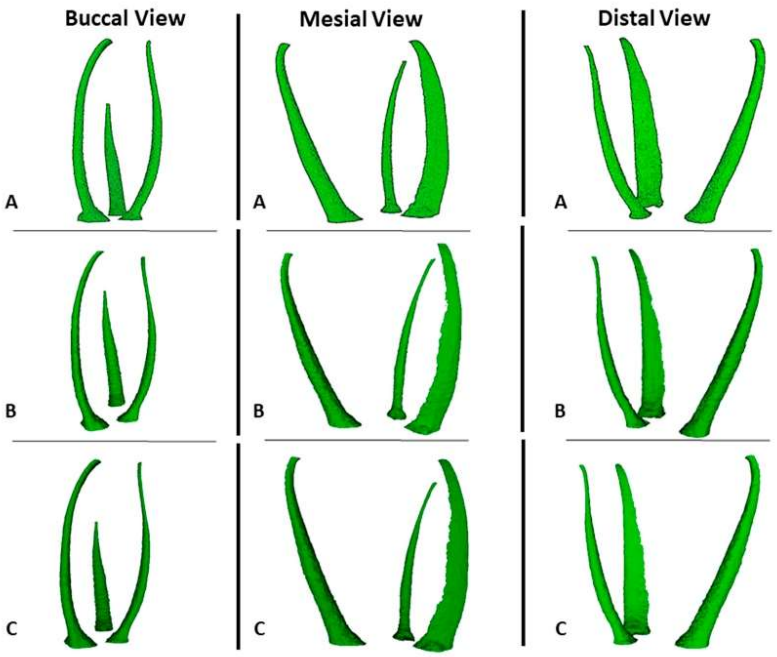

Figure 5. Representative 3D reconstruction of microCT scans after the support material removal protocol and before preparation from different views. (**A**) natural tooth; (**B**) 3D-printed teeth by ProTaper Gold® group; and (**C**) 3D-printed teeth by Endogal® group.

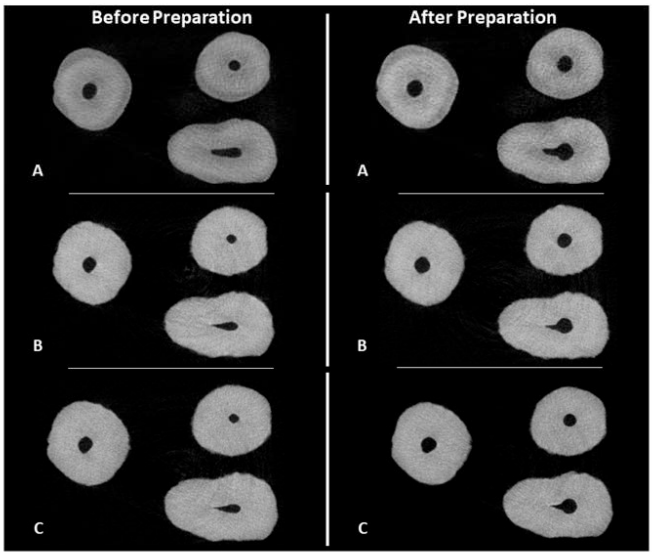

Figure 6. Cross-sectional views of microCT scans before and after preparation. (**A**) natural tooth; (**B**) 3D-printed teeth with ProTaper Gold® group; and (**C**) 3D-printed teeth with Endogal® group.

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Table 2. Microcomputed tomographic analysis before and after preparation with ProTaper Gold® of natural tooth and 3D-printed teeth.

Data		Natural Tooth	3D-Printed Teeth	
Canal Volume (mm^3)	Initial	12.08	Mean ± SD Median	12.12 ± 0.42 12.12
	After	17.75	Mean ± SD Median	17.89 ± 0.69 17.66
	Volume of dentin removed	5.68	Mean ± SD Median	5.77 ± 0.66 5.67
	% Volume increase	46.99	Mean ± SD Median	47.68 ± 6.09 45.50
Centroid X (mm)	Initial	10.92	Mean ± SD Median	10.92 ± 0.07 10.87
	After	11.38	Mean ± SD Median	11.33 ± 0.13 11.32
	Alteration	0.46	Mean ± SD Median	0.41 ± 0.17 0.41
Centroid Y (mm)	Initial	10.37	Mean ± SD Median	10.37 ± 0.07 10.40
	After	10.50	Mean ± SD Median	10.53 ± 0.04 10.53
	Alteration	0.13	Mean ± SD Median	0.16 ± 0.07 0.15
Centroid Z (mm)	Initial	8.03	Mean ± SD Median	8.01 ± 0.11 8.04
	After	8.10	Mean ± SD Median	8.01 ± 0.10 8.02
	Alteration	0.07	Mean ± SD Median	0.01 ± 0.015 0.02
Transportation (mm)		0.48	Mean ± SD Median	0.48 ± 0.14 0.45
Unprepared area (%)		57.64	Mean ± SD Median	54.97 ± 3.79 53.88

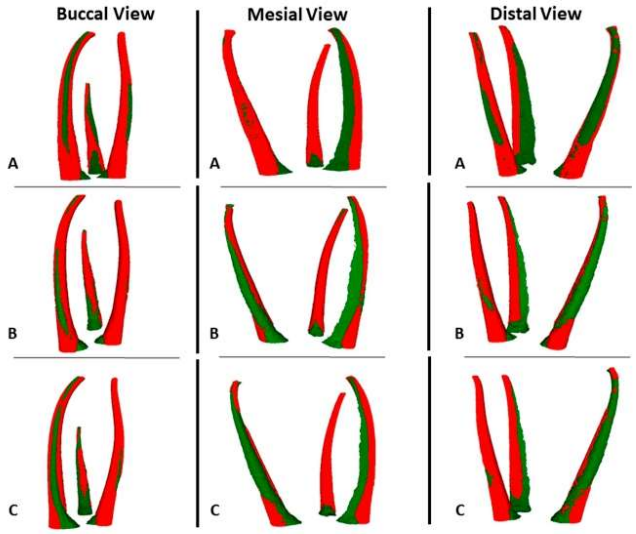

Figure 7. Representative 3D reconstruction of microCT scans before (green) and after (red) preparation from different views. (**A**) natural tooth; (**B**) 3D-printed teeth with ProTaper Gold® group; and (**C**) 3D-printed teeth with Endogal® group.

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Table 3. Microcomputed tomographic analysis before and after preparation with ProTaper Gold® and EndoGal® of 3D-printed teeth.

Data			ProTaper Gold®	
Canal Volume (mm^3)	Initial	Mean ± SD	12.12 ± 0.42	11.86 ± 0.66
		Median	12.12	11.82
	After	Mean ± SD	17.89 ± 0.69	17.02 ± 0.61
		Median	17.66	17.06
	Volume of dentin removed	Mean ± SD	5.77 ± 0.66	5.24 ± 1.02
		Median	5.67	5.49
	% Volume increase	Mean ± SD	47.68 ± 6.09	45.02 ± 10.89
		Median	45.50	47.25
Centroid X (mm)	Initial	Mean ± SD	10.92 ± 0.07	10.92 ± 0.11
		Median	10.87	10.96
	After	Mean ± SD	11.33 ± 0.13	11.37 ± 0.06
		Median	11.32	11.35
	Alteration	Mean ± SD	0.41 ± 0.17	0.45 ± 0.09
		Median	0.41	0.43
Centroid Y (mm)	Initial	Mean ± SD	10.37 ± 0.07	10.33 ± 0.06
		Median	10.40	10.32
	After	Mean ± SD	10.53 ± 0.04	10.44 ± 0.05
		Median	10.53	10.45
	Alteration	Mean ± SD	0.16 ± 0.07	0.11 ± 0.05
		Median	0.15	0.13
Centroid Z (mm)	Initial	Mean ± SD	8.01 ± 0.11	8.03 ± 0.07
		Median	8.04	8.01
	After	Mean ± SD	8.01 ± 0.10	8.07 ± 0.09
		Median	8.02	8.11
	Alteration	Mean ± SD	0.01 ± 0.015	0.05 ± 0.09
		Median	0.02	0.02
Transportation (mm)		Mean ± SD	0.48 ± 0.14	0.49 ± 0.11
		Median	0.45	0.47
Unprepared area (%)		Mean ± SD	54.97 ± 3.79	56.41 ± 5.11
		Median	53.88	55.39

4. Discussion

The present study described an SMRP and compared the root canal anatomy between a NT and 3DPT based on a microCT scan of the NT sample. Comparing the volume and centroids X, Y, and Z of the NT and the 3DPT before preparation, our findings demonstrated no statistically significant differences between them. Therefore, the null hypothesis was accepted. These findings show that the SMRP described is effective in removing the support material SUP706B™ used in this study, and PolyJet™ is adequate for printing 3DPT with similar internal anatomy to NT. The SMRP described here is based on the generic manufacturers' indications for removing the support material SUP706B™ by solubilization, in which an alkaline 2% solution of NaOH and a 1% solution of sodium metasilicate are used in a cleaning station, followed by water rinsing [19]. NaOH is the basis of the SMRP described here, and Derquim® LM 01 alkalin detergent was used, which is a detergent based on NaOH and anionic and non-ionic surfactants, to exert cleaning action on the root canal walls to mimic the detergent function of sodium metasilicate.

Sonic activation with an Endoactivator® was used to improve the irrigants' action by producing intra-canal irrigant agitation and streaming. Since the contact of the Endoactivator® tip with the root canal walls inhibits its free oscillation, reducing irrigant streaming, the tip was placed as deeply as possible without contacting the walls [28,29]. Nevertheless, even if contact occurs, the Endoactivator® tip is a polyamide, which does not produce active root canal cutting, diminishing the risk of root canal anatomy alteration [30]. The Irriflex® needle used is a polyethylene flexible needle that has a smoother progression

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inside the canal and does not wedge against root canal walls. It presents two side openings that produce two jets oriented in the direction of the root canal walls and delivers a large volume of irrigant at a high flow rate with a clinically minimal risk of apical extrusion [31]. However, for the SMRP to be effective, the irrigation must be carried out at high pressure and with the needle tip as close to the apex as possible for irrigant extrusion to occur.

Nevertheless, our findings show differences in the values of the 3DPT before the instrumentation of the parameters evaluated, meaning that the 3DPT are not equal between them. Relative to the root canal anatomy, this could be a result of our protocol since canal patency is mandatory for its effectiveness, being traduced, as stated before, in the visual observation of the extrusion of the irrigants. K-file #15 was chosen for this purpose since in channels under 150 µm, it is very difficult to remove the support material [17], and it was used obligatorily five times for each canal. After this number of utilizations, if the patency was lost, K-file #15 was used to reestablish it. Although used in a passive way, this means that the number of K-file #15 insertions was not the same for all the canals, and its effects on the root canal anatomy should be considered [20,32].

In relation to the total volume of the 3DPT, the alterations in volume that occur with PolyJet™ printing in order to expand have been described [33,34]. The findings of this study show that there is a 0.77% volume expansion, and this can also explain the differences between the 3DPT. The result of the present study is comparable to the 0.71% referred to in a previous study [13]; however, in that study, the STL file of the 3DPT and the volumetric analyses were realised by a 3D scanner in contrast with the microCT methodology of the present study.

Another issue that should be addressed is that, although PolyJet™ has the smallest dimensional error compared to other 3D printing technologies [16,35–37], its accuracy depends on the material used, the geometry of the printed object, and the orientation given in relation to both the printer head and the printer bed [15,34,38]. In a previous study, a rectangular flat part was printed with the long axis parallel to the X, Y, or Z axis of the printer bed, and it was concluded that the long axis of the printed part should be parallel to the X axis [38]. However, to the authors' knowledge, this feature was never studied while printing teeth, and in a single root tooth, the long axis would have been easily defined as the long axis of the root. In the present study, a multiple-root tooth was used, so it was measured as a whole, and the longest distance was in the buccal-to-palatal direction, so this was considered the long axis, and the 3DPT were printed as shown in Figure 2. Nevertheless, future studies should assess which is the best orientation to produce a multiple-root tooth according to the X, Y, or Z axis of the printer bed and if this has relevance to the accuracy of 3DPT. Also, it should be noted that the STL file was simplified and prepared for 3D printing, and this action may also have resulted in some level of distortion [13]. Future studies should assess which level of simplification is supported by the STL file without significant distortion relative to NT.

The NT used in the present study had its crown sectioned and presented an internal anatomy with three single different types of canals; however, it did not present any anatomical irregularities such as lateral canals or isthmus, which are known to be only accessible to irrigants [39]. Future studies are needed to evaluate the effectiveness of the support material in teeth with a full crown and with anatomical irregularities.

Regarding the second aim of this study, in order for 3DPT to be used in ex vivo preparation studies, their behaviour during preparation should be like NT. The present results regarding the volume of dentin removed, the percentage of volume increase, transportation, and unprepared areas show that there were no statistically significant differences between them. Therefore, the null hypothesis was accepted, and it can be concluded that 3DPT behave in a similar way to the NT when prepared with PTG. The major concern expressed about 3DPT is the difference in hardness between resin and human dentin [3,7,9,10,12]. High-temperature RGD525™ is an opaque model material that has exceptional dimensional stability and presents a tensile strength of 70–80 Mpa, a modulus of elasticity of 3200–3500 Mpa, and a flexural strength of 110–130 MPa [40]. In comparison, human dentin presents a tensile strength of

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44.4–97.8 MPa, with a lower value for the inner dentin near to the pulp [41], a modulus of elasticity of 1375–1931 MPa [42], and a flexural strength of 171–254 MPa [43]. All these values between the printing material and dentin are approximated; however, it should be noticed that the material structure differs from the tubular structure of dentin.

Regarding the third aim, the results for the volume of dentin removed, the percentage of volume increase, transportation, and unprepared areas show that there were no statistically significant differences between the two preparation systems. So, the null hypothesis was accepted. Nevertheless, although there are no statistically significant differences, it is observed that ENDG produces a lower volume increase, a lower volume of dentin removed, and a higher percentage of unprepared area, thereby demonstrating that 3DPT are suitable for evaluating the differences between two preparation systems.

To the authors' knowledge, there is no other study that evaluates, using microCT, the shaping properties of the ENDG system, so the present results cannot be compared to others. Regarding the results observed for the PTG system in the present study, the percentage of volume increase was 47.68%, and in the literature, it ranges from 18.7% to 163.32% [22,24,44–46]. The percentage of unprepared area was 54.97%, while in the literature, it ranges from 3.57% to 46.85% [22,24,44–48]. The differences between the present study results and the literature can be explained by the type of teeth or canals used. Most studies use mandibular molar mesial canals; these studies show higher values for the percentage of volume increase and smaller values for the percentage of unprepared area [22,24,45,46], compared to, for instance, studies that use, for example, mandibular incisors or premolars [44,48]. The variation in the canal geometry has an effect on the preparation techniques [49]. It is widely accepted that preparing oval-shaped canals is a challenge, and smaller values for the percentage of unprepared area in this type of canal are associated with brushing movements during preparation and not with pecking movements [50,51].

In the present study, all the preparations were carried out by an experienced dentist in the field of endodontics with clinical experience with the two systems, and the major critical comment of the operator was a higher screw-in effect in the 3DPT compared to the NT, which results in a more difficult preparation, even with a controlled pecking movement of a 2–3 mm amplitude. The screw-in effect is the tendency of a rotary instrument to be pulled into the canal. It is affected by the type of movement kinematics, the cross section, or the taper of the instrument, as well as by the rotational speed [52–55]. In the present study, the manufacturer's recommendations of rotation per minute (rpm) and torque were used. Future studies are needed to establish how changes in rpm, torque, pecking movement amplitude values, and instrument design can influence 3DPT preparation.

As said before, protocols for the standardization of studies using 3DPT still have to be developed [3]. The present study, within its limitations, presents a description of an effective protocol for support material SUP706B™ removal, demonstrating that 3DPT printed with high-temperature RGD525™ material have similar behaviour during endodontic preparation to that of NT. Nonetheless, we propose that future research needs to achieve a standardisation of studies using 3DPT. In summary, the establishment of the optimal orientation regarding the printer bed of multiple-root teeth; the maximal level of simplification of the STL file without losing information if the SMRP described is effective in other types of root morphology; and establishing how changes in the values of the rpm, torque, and amplitude of pecking movements and instrument design can influence the preparation of 3DPT.

5. Conclusions

Within the limitations of the present study, it can be concluded that the SMRP described is effective in removing the support material SUP706B™. PolyJet™ is adequate for printing 3DPT. Furthermore, 3DPT printed with high-temperature RGD525™ have similar behaviour during endodontic preparation with PTG to that of NT, and 3DPT can be used when comparing two preparation systems.

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Data Availability Statement: The data that support the finding of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy and ethical restrictions (undergoing PhD thesis).

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3D printed teeth have the potencial to establish a new standard in endodontic ex vivo studies and endodontic training, potentially supplanting natural teeth. This thesis aimed to identify and try to solve problems that 3D printed teeth in endodontics still present.
The primary aims were (1) to evaluate the impact of support material of a commercial tooth replica, on the preparation technique and its potential effects on the volume increase evaluation using micro-CT scans and, (2) to develop and validate a protocol for complete removing support material from 3D printed teeth and assessing its efficacy by comparing the internal anatomy of a natural tooth with 3D printed teeth using micro-CT.